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(54) **PDGF RECEPTOR KINASE INHIBITORY COMPOUNDS, THEIR PREPARATION, PURIFICATION AND PHARMACEUTICAL COMPOSITIONS INCLUDING SAME**

PDGF-REZEPTOR-KINASE HEMMENDE VERBINDUNGEN, DEREN HERSTELLUNG, REINIGUNG UND DIESE VERBINDUNGEN ENTHALTENDE PHARMAZEUTISCHE ZUSAMMENSETZUNGEN

COMPOSES PDGF INHIBITEURS DU RECEPTEUR DE KINASE, LEUR PREPARATION ET LEUR PURIFICATION, ET COMPOSITIONS PHARMACEUTIQUES A BASE DE CEUX-CI

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WO-A1-98/08848 **WO-A1-99/07701**
WO-A1-99/46264 **WO-A2-99/28304**

- **S. DEMIRAYAK ET. AL.: "Synthesis of some 6,7-disubstituted imidazo[4,5-g]quinoxaline derivatives as possible antimicrobials." ACTA PHARMACEUTICA TURCIA, vol. 40, no. 4, 1998, pages 193-6, XP001145892**

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Description

[0001] The present invention concerns a substantially purified tyrphostin isomer according to claim 1, a pharmaceutical composition according to claim 4, the use of a pharmaceutical composition according to claims 8 and 18, a method of enriching a tyrphostin according to claim 26, a stent according to claim 32.

[0002] The present invention relates to PDGF receptor kinase inhibitory compounds and pharmaceutical compositions such as, but not limited to, slow release compositions. More particularly, the present invention relates to enriched or purified geometrical isomers of compounds of the quinoxaline family known to be PDGF receptor kinase inhibitors, compositions including same, methods of their synthesis, purification and formulation and their use for treatment of proliferative malignant and non-malignant diseases or disorders, such as, but not limited to, psoriasis, hepatic cirrhosis, diabetes, atherosclerosis, restenosis, vascular graft restenosis, in-stent stenosis, angiogenesis, ocular diseases, pulmonary fibrosis, obliterative bronchiolitis, glomerular nephritis, rheumatoid arthritis and PDGF receptor associated malignancies, such as, but not limited to, leukemias and lymphomas.

[0003] Platelet-derived growth factor (PDGF) is a potent mitogen for mesenchymal, glial, and capillary endothelial cells (for reviews, see, [1] and [2]). The three isoforms of PDGF, PDGF-AA, PDGF-AB, and PDGF-BB, interact differentially with structurally related receptors designated PDGF α- and β-receptors. Each of these receptors has an extracellular part featuring five immunoglobulin-like domains, a lipophilic transmembrane domain and an intracellular part with a tyrosine kinase domain containing a characteristic insert amino acid sequence [3-5]. The tyrosine kinase activity of these receptors is essential for transmission of the mitogenic signal into the cell [6].

[0004] PDGF and its receptors participate in various physiological processes such as embryonal development and wound healing: An abnormally high activity of PDGF is believed to play a central role in the etiology of certain adverse pathophysiological situations, such as atherosclerosis and restenosis [7, 8], as well as in other non-malignant diseases such as pulmonary fibrosis [9], glomerular nephritis [10], and rheumatoid arthritis [11]. Moreover, the PDGF B-chain was acquired as the *sis* oncogene by the acutely transforming simian sarcoma virus [12, 13]. The expression of a PDGF-like growth factor in cells infected with simian sarcoma virus or transfected with the *sis* oncogene leads to their transformation due to the persistent autocrine stimulation of the resident PDGF receptors.

[0005] Furthermore, certain human tumors possess PDGF receptors and express the genes for PDGF which suggest that autocrine growth stimulation via PDGF receptors contributes to the malignant phenotype of these tumors [2, 14].

[0006] The fact that PDGF is likely to be involved in the development of certain disorders has prompted the search for agents to block the action of PDGF. The approaches for interference with PDGF-induced signalling include peptides competing with PDGF for receptor binding [15], dominant negative mutants of PDGF [16, 17] or of PDGF receptor [18], and low molecular weight blockers of the receptor tyrosine kinase activity known as tyrphostins [19, WO-A-99 07701].

[0007] Certain tyrphostins which block PDGF-dependent proliferation of rabbit vascular smooth muscle cells [20] and of human bone marrow fibroblasts [21] have already been reported.

[0008] A novel class of tyrosine kinase blockers represented by the tyrphostins AG1295 and AG1296 was described by Kovalenko *et al.* [22]. These compounds inhibit selectively the platelet-derived growth factor (PDGF) receptor kinase and the PDGF dependent DNA synthesis in Swiss 3T3 cells and in porcine aorta endothelial cells (EC) with 50 % inhibitory concentrations below 5 and 1 μM, respectively. These PDGF receptor blockers have no effect on epidermal growth factor receptor autophosphorylation, weak effects on DNA synthesis stimulated by insulin, by epidermal growth factor, or by a combination of both and over an order of magnitude weaker blocking effect on fibroblast growth factor-dependent DNA synthesis.

[0009] AG1296 potently inhibits signalling of human PDGF α- and β-receptors as well as of the related stem cell factor receptor (c-Kit) but has no effect on autophosphorylation of the vascular endothelial growth factor receptor KDR or on DNA synthesis induced by vascular endothelial growth factor in porcine aortic endothelial cells. Treatment by AG1296 reverses the transformed phenotype of sis-transfected NIH 3T3 cells but has no effect on *src*-transformed NIH 3T3 cells or on the activity of the kinase p60c-*src*(F527) immunoprecipitated from these cells [22].

[0010] In U.S.-A-5,932,580 filed December 1, 1997, further low molecular weight PDGF receptor kinase inhibitors, of the quinoxaline family, are described. Specifically, substituted analogs of 1,2-dimethylimidazolo[5,4-g]quinoxaline were shown to selectively inhibit PDGFR autophosphorylations and proliferation of PDGFR expressing cells, like porcine arterial smooth muscle cells (SMC), porcine endothelial cells and human internal mammary artery SMC, at μM concentration range.

[0011] Acta Pharmaceutica Turcia, vol. 40, p. 193-6 (1998) describe synthesis of some 6,7-disubstituted imidazo [4,5-g]quinoxaline derivatives as possible antimicrobials.

[0012] WO-A-99/07701 relates to tricyclic quinoxaline compounds and physiologically acceptable salts and prodrugs thereof which modulate the activity of protein tyrosine kinases and therefore should be useful in the prevention and treatment of protein tyrosine kinase related cellular disorders such as cancer.

[0013] WO-A-99/28304 concerns PDGF receptor kinase inhibitory compounds of the quinoxaline family, methods for their synthesis and containment in slow release pharmaceutical preparations, and their use for treatment of prolif-

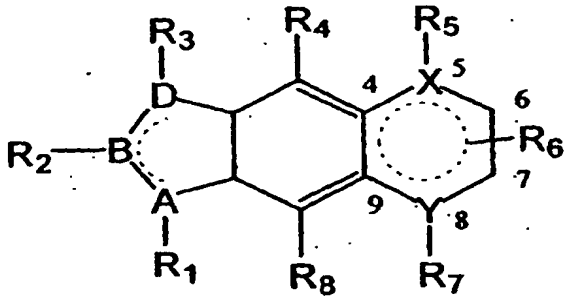
erative malignant and non-malignant diseases or disorders by local or systemic application. A compound according to the invention includes a tyrphostin of general formula (I), wherein R1 and R2 are each independently selected from the group consisting of alkyl, halogen, nitro and amine and Ar is selected from the group consisting of phenyl, ferrocene, thiophene, furane, pyrrole, indole, thiazole, imidazole and pyridine.

[0014] The present invention describes enriched or purified geometrical isomers of compounds of the quinoxaline family known to be PDGF receptor kinase inhibitors, compositions including same, methods of their synthesis, purification and formulation and their use for treatment of proliferative malignant and non-malignant diseases or disorders, which show differential selectivity towards the PDGF receptor kinase. It is shown herein for the first time that geometrical isomers of compounds belonging to the quinoxaline are producable, isomerically purifyable and have differential affinity towards PDGF receptor kinase.

[0015] The substantially purified tyrphostin isomer is defined in claim 1, the pharmaceutical compostiion is defined in claim 4, the use of a pharmaceutical composition is defined in claims 8 and 18, the method of enriching a tyrphostin is defined in claim 26, the stent is defined in claim 32.

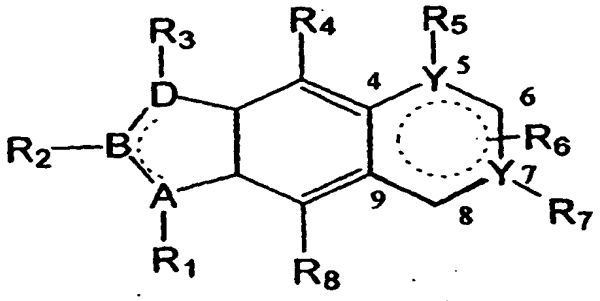
[0016] It is an object of the present invention to provide PDGF receptor kinase inhibitory compounds of the quinoxaline family, methods for their synthesis and purification and containment in, for example, slow release pharmaceutical compositions, and their use for treatment of a variety of diseases and disorders by local or systemic application.

[0017] According to one aspect of the present invention there is provided a preparation of a tyrphostin comprising a compound of a general formula:



(Compound I)

or



(Compound II)

wherein,

4, 5, 6, 7, 8 and 9 indicate positions on a terminal 6-member ring;

A, B, D, X and Y are each nitrogen,

R_1, R_2, R_3, R_5 and R_7 are each independently selected from the group consisting of hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, aryl, hydroxy, alkoxy, halo, C-carboxy, O-carboxy, carbonyl, thiocarbonyl, C-amido, guanyl, sulfonyl, trihalomethane-sulfonyl and a pair of electrons, or alternatively, R_1 and R_2 or R_2 and R_3 form a 5-7 member ring structure;

R_6 is selected from the group consisting of alkyl, trihaloalkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, sulfinyl, sulfonyl, N-sulfonamido, S-sulfonami-

do, trihalomethylsulfonamido, carbonyl, thiocarbonyl, C-carboxy, O-carboxy, C-amido, N-amido, cyano, nitro, halo, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, ureido, guanyl, guanidino, amino and a physiologically acceptable salt or a prodrug thereof;

R_4 and R_8 are each independently selected from the group consisting of hydrogen, alkyl, trihaloalkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, sulfinyl, sulfonyl, N-sulfonamido, S-sulfonamido, trihalomethylsulfonamido, carbonyl, thiocarbonyl, C-carboxy, O-carboxy, C-amido, N-amido, cyano, nitro, halo, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, ureido, guanyl, guanidino, amino and - $NR_{10}R_{11}$ and, a physiologically acceptable salt or a prodrug thereof;

R_{10} and R_{11} are each independently selected from the group consisting of hydrogen, alkyl, cycloalkyl, aryl, carbonyl and sulfonyl, or alternatively R_{10} and R_{11} form a five- or six-member heteroalicyclic ring; and, a physiologically acceptable salt or a prodrug thereof;

whereas, R_6 at position 6.

[0018] According to further features in preferred embodiments of the invention described below, A, D, X and Y are each a nitrogen; B is a carbon; R_1 and R_2 are each independently selected from the group consisting of alkyl, alkoxy, halogen, nitro and amine group; R_3, R_5 and R_7 are each a pair of electrons; R_6 is an aryl, selected from the group consisting of phenyl, ferrocene, thiophene, furane, pyrrole, indole, thiazole, imidazole and pyridine.

[0019] According to still further features in the described preferred embodiments
R_1 and R_2 are each a methyl; R_4 and R_8 are each a hydrogen.

[0020] According to still further features in the described preferred embodiments the preparation is enriched for Compound I in which R_6 is at position 6.

[0021] According to still further features in the described preferred embodiments the preparation is enriched for Compound II in which R_6 is at position 6.

[0022] According to another aspect of the present invention there is provided a pharmaceutical composition comprising, as an active ingredient, the preparation described herein and a pharmaceutically acceptable carrier.

[0023] According to further features in preferred embodiments of the invention described below, the pharmaceutically acceptable carrier is a slow release carrier.

[0024] According to still further features in the described preferred embodiments the slow release carrier is polylactic acid.

[0025] According to yet another aspect of the present invention there is provided a method of treating or preventing a protein tyrosine kinase related disorder in an organism, the method comprising the step of administering to the organism a therapeutically effective amount of the pharmaceutical composition described herein.

[0026] According to further features in preferred embodiments of the invention described below, the protein tyrosine kinase related disorder is selected from the group consisting of an EGF related disorder, a PDGF related disorder, an IGF related disorder and a met related disorder.

[0027] According to still further features in the described preferred embodiments the protein tyrosine kinase related disorder is selected from the group consisting of a cell proliferative disorder, a fibrotic disorder and a metabolic disorder.

[0028] According to still further features in the described preferred embodiments the cell proliferative disorder is selected from the group consisting of papilloma, blastoglioma, Kaposi's sarcoma, melanoma, lung cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, astrocytoma, head cancer, neck cancer, bladder cancer, breast cancer, lung cancer, colorectal cancer, thyroid cancer, pancreatic cancer, gastric cancer, hepatocellular carcinoma, leukemia, lymphoma, Hodgkin's disease, Burkitt's disease, arthritis, rheumatoid arthritis, diabetic retinopathy, angiogenesis, restenosis, in-stent restenosis, vascular graft restenosis.

[0029] According to still further features in the described preferred embodiments the cell fibrotic disorder is selected from the group consisting of pulmonary fibrosis, hepatic cirrhosis, atherosclerosis, glomerulonephritis, diabetic nephropathy, thrombic microangiopathy syndromes, transplant rejection.

[0030] According to still further features in the described preferred embodiments the cell metabolic disorder is selected from the group consisting of psoriasis, diabetes, wound healing, inflammation, and neurodegenerative diseases.

[0031] According to still further features in the described preferred embodiments the organism is a mammal.

[0032] According to still further features in the described preferred embodiments the mammal is a human.

[0033] According to still another aspect of the present invention there is provided a method of locally treating or preventing a disorder of a tissue of an organism comprising the step of locally applying the pharmaceutical composition described herein onto the tissue.

[0034] According to further features in preferred embodiments of the invention described below, the tissue is selected from the group consisting of blood vessel, lung and skin.

[0035] According to an additional aspect of the present invention there is provided a method of inhibiting cell proliferation comprising the step of subjecting the cells to the tyrphostin preparation described herein.

[0036] According to further features in preferred embodiments of the invention described below, the cells are of an organism, whereas subjecting the cells to the preparation is effected *in vivo* or *in vitro*.

[0037] According to yet an additional aspect of the present invention there is provided a method of enriching a preparation of tyrphostins for a specific geometrical isomer, the method comprising the steps of (a) chromatographing the preparation through a matrix, thereby separating isomers in the preparation; (b) collecting at least one specific isomer. Optionally, the method further comprising the step of (c) crystallizing the at least one specific isomer.

[0038] According to still an additional aspect of the present invention there is provided a method for preparing a pharmaceutical composition for slow release of a tyrphostin comprising the steps of (a) providing an isomer-enriched tyrphostin preparation as described herein; (b) dissolving or dispersing a slow release carrier and the isomer-enriched tyrphostin preparation in an organic solvent for obtaining an organic solution containing the carrier and the isomer-enriched tyrphostin preparation; (c) adding the organic solution into an aqueous solution for obtaining an oil-in-water-type emulsion; and (d) evaporating the organic solvent from the oil-in-water-type emulsion for obtaining a colloidal suspension of particles containing the slow release carrier and the isomer-enriched tyrphostin preparation.

[0039] According to further features in preferred embodiments of the invention described below, the slow release carrier is polylactic acid.

[0040] According to a further aspect of the present invention there is provided a stent comprising a substantially tubular body, the body is made of a material designed for slow release of a tyrphostin preparation as described herein.

[0041] The present invention successfully addresses the shortcomings of the presently known configurations by providing new and potent tyrphostins and delivery system for treatment of a variety of disorders and diseases.

[0042] The invention is herein described, by way of example only, with reference to the accompanying -illustrations, wherein:

FIG. 1 shows two perspectives of a unit cell crystal structure of purified geometrical isomer AG2043 (1,2-dimethyl-6-(2-thiophene)imidazolo[5,4-g] quinoxaline);

FIGs. 2a-b show the molecular structure of geometrical isomer AG2043 (1,2-dimethyl-6-(2-thiophene)imidazolo [5,4-g] quinoxaline) according to the present invention;

FIG. 3 shows two perspectives of a unit cell crystal structure of purified geometrical isomer AG2044 (1,2-dimethyl-7-(2-thiophene)imidazolo[5,4-g] quinoxaline);

FIG. 4a-b show the molecular structure of geometrical isomer AG2044 (1,2-dimethyl-7-(2-thiophene)imidazolo [5,4-g] quinoxaline) according to the present invention;

FIG. 5 presents radiograms demonstrating inhibition of PDGFR autophosphorylation in a cell free system derived from Swiss 3T3 cell membranes by AG2033 (1,2-dimethyl-6-phenyl imidazolo[5,4-g] quinoxaline) and AG2043 (1,2-dimethyl-6-(2-thiophene)imidazolo[5,4-g] quinoxaline) purified isomers;

FIG. 6 shows a dose-response curve for purified AG2033 (1,2-dimethyl-6-phenyl imidazolo[5,4-g] quinoxaline) inhibitory effect on PDGFR autophosphorylation;

FIG. 7 shows a dose-response curve for purified AG2043 (1,2-dimethyl-6-(2-thiophene)imidazolo[5,4-g] quinoxaline) inhibitory effect on PDGFR autophosphorylation;

FIG. 8 presents radiograms demonstrating inhibition of PDGFR autophosphorylation in intact Swiss 3T3 cells comparing each isomer pair, AG2033 and AG2034 (1,2-dimethyl-6-phenyl imidazolo[5,4-g] quinoxaline, (1,2-dimethyl-7-phenyl imidazolo[5,4-g] quinoxaline, respectively); AG2043 and AG2044 (1,2-dimethyl-6-(2-thiophene)imidazolo [5,4-g] quinoxaline, (1,2-dimethyl-7- (2-thiophene)imidazolo[5,4-g] quinoxaline, respectively);

FIG. 9 presents plots demonstrating the inhibitory and recovery effects of purified AG2043 and AG2044 isomers (1,2-dimethyl-6-(2-thiophene)imidazolo[5,4-g] quinoxaline, (1,2-dimethyl-7- (2-thiophene)imidazolo[5,4-g] quinoxaline, respectively) on porcine SMC proliferation.

FIG. 10 is a bar graph, demonstrating the inhibitory effect of purified AG2033 isomer (1,2-dimethyl-6-phenyl imidazolo[5,4-g] quinoxaline) on human coronary artery SMC (HCASMC) migration; and

FIG. 11 is a bar graph, demonstrating the inhibitory effect of purified AG2043 isomer (1,2-dimethyl-6-(2-thiophene) imidazolo[5,4-g] quinoxaline) on human coronary artery SMC (HCASMC) migration.

[0043] The present invention is of quinoxaline derivatives, isomers of which modulate the activity of protein tyrosine kinases (PTKs). These may include disorders associated with tyrosine kinase receptors, such as, but not limited to, PDGFR, EGFR, IGFR, and FGFR. More specifically, the present invention is of enriched or purified geometrical isomers of compounds of the quinoxaline family known to be PDGF receptor kinase inhibitors, compositions including same, methods of their synthesis, purification and formulation and their use for treatment of proliferative malignant and non-malignant diseases, fibrotic or metabolic disorders, such as, but not limited to, psoriasis, hepatic cirrhosis, diabetes, atherosclerosis, restenosis, vascular graft restenosis, in-stent stenosis, angiogenesis, ocular diseases, pulmonary fibrosis, glomerular nephritis, and rheumatoid arthritis, and PDGF receptor associated malignancies, such as, but not limited to, leukemias and lymphomas, by local or systemic application of the disclosed preparations and compositions.

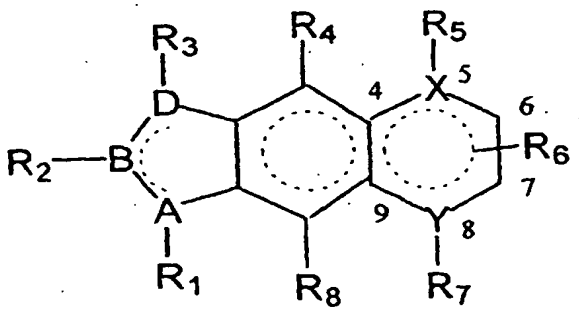
[0044] While conceiving the present invention, it was realized that the outcome of the chemical synthetic procedure of certain quinoxalines includes several isomeric products of the quinoxaline compound. More specifically, substitution

on the terminal 6-member ring can assume two alternative positions. Thus, potential differences in specific isomer potency and selectivity were hypothesized, which may result in a differential blockade of PDGF receptor activation and consequent inhibition of, for example, SMC activation, migration and proliferation.

[0045] The experiments described below in the Examples section demonstrate that the two possible isomers are indeed formed, and are separable. Additionally, it is shown that tyrphostin-mediated inhibition of the PDGF receptor autophosphorylation results in the selective inhibition of SMC and PDGFR-expressing-PAEC cell proliferation and migration, *in vitro,* with a minimal inhibitory effect on KDR-expressing-PAEC cells. It is further shown below that the purified geometrical tyrphostin isomers AG2033 and AG2043 exhibit higher potency in completely blocking PDGF-BB induced phosphorylation of PDGF-β-R and consequent proliferation, relative to their isomeric counterparts, AG2034 and AG2044, respectively.

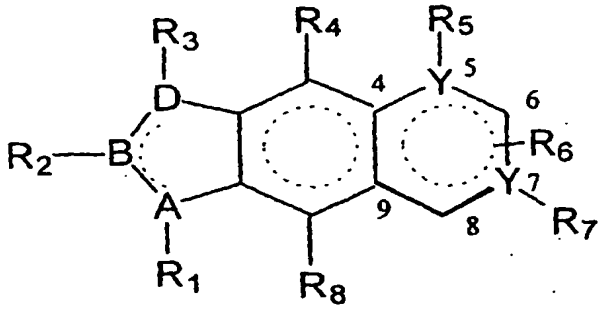
Tyrphostins-containing-preparations:

[0046] Thus, a preparation according to the present invention includes any mixture of synthetic tyrphostin products of the general formula:



(Compound I)

or



(Compound II)

4, 5, 6, 7, 8 and 9 indicate positions on a terminal 6-member ring.

[0047] The doted lines indicate aromatic system.

[0048] A, B, D, X and Y are each independently a carbon, nitrogen, oxygen or sulfur.

[0049] R_1, R_2, R_3, R_5 and R_7 are each independently a hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, aryl, hydroxy, alkoxy, halo, C-carboxy, O-carboxy, carbonyl, thiocarbonyl, C-amido, guanyl, sulfonyl, trihalomethane-sulfonyl and a pair of electrons, or alternatively, R_1 and R_2 or R_2 and R_3 form a 5-7 member ring structure.

[0050] R_6 is alkyl, trihaloalkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, sulfinyl, sulfonyl, N-sulfonamido, S-sulfonamido, trihalomethylsulfonamido, carbonyl, thiocarbonyl, C-carboxy, O-carboxy, C-amido, N-amido, cyano, nitro, halo, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, ureido, guanyl, guanidino, amino or a physiologically acceptable salt or a prodrug thereof.

[0051] R_4 and R_8 are each independently hydrogen, alkyl, trihaloalkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, cyano, nitro, azido, carbonyl, thioalkoxy, thioaryloxy, sulfinyl, sulfonyl, N-sulfonamido, S-sulfonamido, trihalomethylsulfonamido, carbonyl, thiocarbonyl, C-carboxy, O-carboxy, C-amido, N-amido, cyano, nitro, halo, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, ureido, guanyl, guanidino, amino and $-NR_{10}R_{11}$ and, a physiologically acceptable salt or a prodrug thereof.

[0052] R_{10} and R_{11} are each independently hydrogen, alkyl, cycloalkyl, aryl, carbonyl and sulfonyl, or alternatively R_{10} and R_{11} form a five- or six-member heteroalicyclic ring and, a physiologically acceptable salt or a prodrug thereof.

[0053] Additional examples for R_6 substituents are found in WO-A-99 07701 (see Tables 1 and 2, on pages 20-27 and 34-35).

[0054] The preparation according to the present invention is enriched either for R_6 at position 6 or for R_6 at position 7 for Compound I, or the preparation is enriched either for R_6 at position 6 or for R_6 at position 8 for Compound II.

[0055] As used herein in the specification and in the claims section that follows, the term "prodrug" refers to an agent which is converted into an active parent drug *in vivo*. Prodrugs are often useful because in some instances they may be easier to administer than the parent drug. They may, for instance, be bioavailable by oral administration whereas the parent drug is not. The prodrug may also have improved solubility compared to the parent drug in pharmaceutical compositions. An example, without limitation, of a prodrug would be a compound of the present invention which is administered as an ester (the "prodrug") to facilitate transmittal across a cell membrane where water solubility is not beneficial, but which then is metabolically hydrolyzed to the carboxylic acid once inside the cell where water solubility is beneficial.

[0056] As used herein in the specification and in the claims section that follows, the term "ester" refers to a -C-OO-R" group, where R" is alkyl, cycloalkyl, aryl, heteroaryl (bonded through a ring carbon) or heteroalicyclic (bonded through a ring carbon).

[0057] As used herein in the specification and in the claims section that follows, the phrase "physiologically acceptable salt" refers to a charged species of the tyrphostin compound and its counter ion, so that it does not cause significant irritation to an organism and does not abrogate the biological activity and properties of the administered compound.

[0058] As used herein in the specification and in the claims section that follows, the phrase "enriched isomer preparation" refers to a preparation in which one isomer is represented in a higher proportion as compared to its synthesis proportion.

[0059] As used herein in the specification and in the claims section that follows, the term "alkyl" refers to a saturated aliphatic hydrocarbon including straight chain and branched chain groups. Preferably, the alkyl group has 1 to 20 carbon atoms. Whenever a numerical range; e.g., "1-20", is stated herein, it means that the group, in this case the alkyl group, may contain 1 carbon atom, 2 carbon atoms, 3 carbon atoms, etc., up to and including 20 carbon atoms. More preferably, it is a medium size alkyl having 1 to 10 carbon atoms. Most preferably, it is a lower alkyl having 1 to 4 carbon atoms. The alkyl group may be substituted or unsubstituted. When substituted, the substituent group can be, for example, cycloalkyl, aryl, heteroaryl, heteroalicyclic, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, cyano, nitro, azido, carbonyl, thioalkoxy, thioaryloxy, cyano, halo, carbonyl, thiocarbonyl, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, C-carboxy, O-carboxy, nitro, sulfonamido, trihalomethanesulfonamido, silyl, guanyl, guanidino, ureido, amino or $NR_{10}R_{11}$, wherein R_{10} and R_{11} are each independently hydrogen, alkyl, cycloalkyl, aryl, carbonyl, sulfonyl, trihalomethysulfonyl and, combined, a five- or six-member heteroalicyclic ring.

[0060] A "cycloalkyl" group refers to an all-carbon monocyclic or fused ring (i.e., rings which share an adjacent pair of carbon atoms) group wherein one of more of the rings does not have a completely conjugated pi-electron system. Examples, without limitation, of cycloalkyl groups are cyclopropane, cyclobutane, cyclopentane, cyclopentene, cyclohexane, cyclohexadiene, cycloheptane, cycloheptatriene, and adamantane. A cycloalkyl group may be substituted or unsubstituted. When substituted, the substituent group can be, for example, alkyl, aryl, heteroaryl, heteroalicyclic, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, cyano, nitro, azido, carbonyl, thioalkoxy, thioaryloxy, cyano, halo, carbonyl, thiocarbonyl, C-carboxy, O-carboxy, O-carbamyl, N-carbamyl, C-amido, N-amido, nitro, amino and $NR_{10}R_{11}$ as defined above.

[0061] An "alkenyl" group refers to an alkyl group which consists of at least two carbon atoms and at least one carbon-carbon double bond. An "alkynyl" group refers to an alkyl group which consists of at least two carbon atoms and at least one carbon-carbon triple bond

[0062] An "aryl" group refers to an all-carbon monocyclic or fused-ring polycyclic (i.e., rings which share adjacent pairs of carbon atoms) groups having a completely conjugated pi-electron system. Examples, without limitation, of aryl groups are phenyl, naphthalenyl and anthracenyl. The aryl group may be substituted or unsubstituted. When substituted, the substituent group can be, for example, halo, trihalomethyl, alkyl, hydroxy, alkoxy, aryloxy, thiohydroxy, thiocarbonyl, C-carboxy, O-carboxy, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, sulfinyl, sulfonyl, amino and $NR_{10}R_{11}$ as defined above.

[0063] A "heteroaryl" group refers to a monocyclic or fused ring (i.e., rings which share an adjacent pair of atoms) group having in the ring(s) one or more atoms, such as, for example, nitrogen, oxygen and sulfur and, in addition,

having a completely conjugated pi-electron system. Examples, without limitation, of heteroaryl groups include pyrrole, furane, thiophene, imidazole, oxazole, thiazole, pyrazole, pyridine, pyrimidine, quinoline, isoquinoline and purine. The heteroaryl group may be substituted or unsubstituted. When substituted, the substituent group can be, for example, alkyl, cycloalkyl, halo, trihalomethyl, hydroxy, alkoxy, aryloxy, thiohydroxy, thiocarbonyl, sulfonamido, C-carboxy, O-carboxy, sulfinyl, sulfonyl, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, amino or $NR_{10}R_{11}$ as defined above.

[0064] A "heteroalicyclic" group refers to a monocyclic or fused ring group having in the ring(s) one or more atoms such as nitrogen, oxygen and sulfur. The rings may also have one or more double bonds. However, the rings do not have a completely conjugated pi-electron system. The heteroalicyclic may be substituted or unsubstituted. When substituted, the substituted group can be, for example, alkyl, cycloalkyl, aryl, heteroaryl, halo, trihalomethyl, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, cyano, nitro, carbonyl, thiocarbonyl, C-carboxy, O-carboxy, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, sulfinyl, sulfonyl, C-amido, N-amido, amino and $NR_{10}R_{11}$ as defined above.

[0065] A "hydroxy" group refers to an -OH group.

[0066] An "azido" group refers to a -N=N group.

[0067] An "alkoxy" group refers to both an -O-alkyl and an -O-cycloalkyl group, as defined herein.

[0068] An "aryloxy" group refers to both an -O-aryl and an -O-heteroaryl group, as defined herein.

[0069] A "thiohydroxy" group refers to an -SH group.

[0070] A "thioalkoxy" group refers to both an -S-alkyl group, and an -S-cycloalkyl group, as defined herein.

[0071] An "thioaryloxy" group refers to both an -S-aryl and an -S-heteroaryl group, as defined herein.

[0072] A "carbonyl" group refers to a -C(=O)-R" group, where R" is hydrogen, alkyl, cycloalkyl, aryl, heteroaryl (bonded through a ring carbon) or heteroalicyclic (bonded through a ring carbon) as defined herein.

[0073] An "aldehyde" group refers to a carbonyl group, where R" is hydrogen.

[0074] A "thiocarbonyl" group refers to a -C(=S)-R" group, where R" is as defined herein.

[0075] A "C-carboxy" group refers to a -C(=O)-O-R" groups, where R" is as defined herein.

[0076] An "O-carboxy" group refers to an R"C(=O)-O- group, where R" is as defined herein.

[0077] A "carboxylic acid" group refers to a C-carboxyl group in which R" is hydrogen.

[0078] A "halo" group refers to flourine, chlorine, bromine or iodine.

[0079] A "trihalomethyl" group refers to a -CX group wherein X is a halo group as defined herein.

[0080] A "trihalomethanesulfonyl" group refers to an $X_3CS(=O)_2$- group wherein X is a halo group as defined herein.

[0081] A "sulfinyl" group refers to an -S(=O)-R" group, where R" is as defined herein.

[0082] A "sulfonyl" group refers to an $-S(=O)_2-R$" group, where R" is as defined herein.

[0083] An "S-sulfonamido" group refers to a $-S(=O)_2-NR_{10}R_{11}$ group, with R_{10} and R_{11} as defined herein.

[0084] An "N-sulfonamido" group refers to an $R_{10}(=O)_2-NR_{11}$ group, where R_{10} and R_{11} are as defined herein.

[0085] A "trihalomethanesulfonamido" group refers to an $X_3CS(=O)_2NR_{10}$-group, where R_{10} is as defined herein.

[0086] An "O-carbamyl" group refers to an $-OC(=O)-NR_{10}R_{11}$ group, where R_{10} and R_{11} are as defined herein.

[0087] An "N-carbamyl" group refers to an $R_{11}OC(=O)-NR_{10}$- group, where R_{10} and R_{11} are as defined herein.

[0088] An "O-thiocarbamyl" group refers to an $-OC(=S)-NR_{10}R_{11}$ group, where R_{10} and R_{11} are as defined herein.

[0089] An "N-thiocarbamyl" group refers to an $R_{11}OC(=S)NR_{10}$- group, where R_{10} and R_{11} are as defined herein.

[0090] An "Amino" group refers to an $-NH_2$ group. A "C-amido" group refers to a $-C(=O)-NR_{10}R_{11}$ group, where R_{10} and R_{11} are as defined herein.

[0091] An "N-amido" group refers to an $R_{11}C(=O)-NR_{10}$ group, where R_{10} and R_{11} are as defined herein.

[0092] A "quaternary ammonium" group refers to an $-NHR_{10}R_{11}$ group, wherein R_{10} and R_{11} are independently alkyl, cycloalkyl, aryl or heteroaryl.

[0093] An "ureido" group refers to an $-NR_{10}C(=O)-NR_{11}R_{12}$ group, where R_{10} and R_{11} are as defined herein and R_{12} is defined as either R_{10} or R_{11}.

[0094] A "guanidino" group refers to an $-R_{10}NC(=N)-NR_{11}R_{12}$ group, where R_{10}, R_{11} and R_{12} are as defined herein.

[0095] A "guanyl" group refers to an $R_{10}R_{11}NC(=N)$- group, where R_{10} and R_{11} are as defined herein.

[0096] A "nitro" group refers to an $-NO_2$ group.

[0097] A "cyano" group refers to a -C=N group.

[0098] A "silyl" group refers to a -Si $(R")_3$, where R" is as defined herein.

[0099] According to a preferred embodiment of the present invention A, D, X and Y are each a nitrogen. B is a carbon; R_1 and R_2 are each independently alkyl, alkoxy, halogen, nitro and amine group; R_3, R_5 and R_7 are each a pair of electrons and R_6 is an aryl such as phenyl, ferrocene, thiophene, furane, pyrrole, indole, thiazole, imidazole or pyridine.

[0100] According to a further preferred embodiment of the present invention wherein R_1 and R_2 are each a methyl, and R_4 and R_8 are each a hydrogen.

[0101] Herein the term "purified isomer preparation" refers to a preparation consisting of substantially 100 % of a single isomer type.

Isomer enrichment:

[0102] Further according to the present invention there is provided a method of enriching the preparation of tyrphostins for a specific geometrical isomer as herein described. The method includes the following steps:

First, the preparation is chromatographed through a matrix, thereby a separation of the different isomers is achieved.
Second, fractions from the chromatography are collected, such that at least one specific isomer is obtained.
Third, an optional step of crystallizing a specific isomer can be effected to achieve 100 % purity in a crystal structure.

Pharmaceutical compositions:

[0103] Further according to the present invention there is provided a pharmaceutical composition including a tyrphostin preparation as described hereinabove as an active ingredient. The preparation according to the present invention can be administered to an organism *per se,* or in a pharmaceutical composition where it is mixed with suitable carriers or excipients.

[0104] As used herein a "pharmaceutical composition" refers to a preparation of one or more of the isomeric compounds described herein, or physiologically acceptable salts or prodrugs thereof, with other chemical components such as physiologically suitable carriers and excipients. The purpose of a pharmaceutical composition is to facilitate administration of a compound to an organism.

[0105] Herein the term "active ingredient" refers to the tyrphostin preparation or compound accountable for the biological effect.

[0106] Hereinafter, the terms "physiologically acceptable carrier" and "pharmaceutically acceptable carrier" which may be interchangeably used refer to a carrier or a diluent that does not cause significant irritation to an organism and does not abrogate the biological activity and properties of the administered compound.

[0107] Herein the term "excipient" refers to an inert substance added to a pharmaceutical composition to further facilitate administration of a compound. Examples, without limitation, of excipients include calcium carbonate, calcium phosphate, various sugars and types of starch, cellulose derivatives, gelatin, vegetable oils and polyethylene glycols.

[0108] Techniques for formulation and administration of drugs may be found in "Remington's Pharmaceutical Sciences," Mack Publishing Co., Easton, PA, latest edition, which is incorporated herein by reference.

[0109] ***Routes of administration***: Suitable routes of administration may, for example, include oral, rectal, transmucosal, intestinal or parenteral delivery, including intramuscular, subcutaneous and intramedullary injections as well as intrathecal, direct intraventricular, intravenous, inrtaperitoneal, intranasal, or intraocular injections.

[0110] Alternately, one may administer a tyrphostin preparation in a local rather than systemic manner, for example, via injection of the preparation directly into a solid tumor often in a depot or slow release formulation, such as described below.

[0111] Furthermore, one may administer the drug in a targeted drug delivery system, for example, in a liposome coated with a tumor specific antibody. The liposomes will be targeted to and taken up selectively by the tumor.

[0112] ***Composition/formulation***: Pharmaceutical compositions of the present invention may be manufactured by processes well known in the art, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes.

[0113] Pharmaceutical compositions for use in accordance with the present invention thus may be formulated in conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries, which facilitate processing of the active compounds into preparations which, can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen.

[0114] For injection, the compounds of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hank's solution, Ringer's solution, or physiological saline buffer. For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

[0115] For oral administration, the compounds can be formulated readily by combining the active compounds with pharmaceutically acceptable carriers well known in the art. Such carriers enable the compounds of the invention to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for oral ingestion by a patient. Pharmacological preparations for oral use can be made using a solid excipient, optionally grinding the resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carbomethylcellulose; and/or physiologically acceptable polymers such as polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as

cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

[0116] Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, titanium dioxide, lacquer solutions and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

[0117] Pharmaceutical compositions, which can be used orally, include push-fit capsules made of gelatin as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules may contain the active ingredients in admixture with filler such as lactose, binders such as starches, lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. All formulations for oral administration should be in dosages suitable for the chosen route of administration.

[0118] For buccal administration, the compositions may take the form of tablets or lozenges formulated in conventional manner.

[0119] For administration by inhalation, the compounds for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation from a pressurized pack or a nebulizer with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichloro-tetrafluoroethane or carbon dioxide. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, e.g., gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

[0120] The preparations described herein may be formulated for parenteral administration, e.g., by bolus injection or continuos infusion. Formulations for injection may be presented in unit dosage form, e.g., in ampoules or in multidose containers with optionally, an added preservative. The compositions may be suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents.

[0121] Pharmaceutical compositions for parenteral administration include aqueous solutions of the active preparation in water-soluble form. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acids esters such as ethyl oleate, triglycerides or liposomes. Aqueous injection suspensions may contain substances, which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol or dextran. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

[0122] Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g., sterile, pyrogen-free water, before use.

[0123] The preparation of the present invention may also be formulated in rectal compositions such as suppositories or retention enemas, using, e.g., conventional suppository bases such as cocoa butter or other glycerides.

[0124] In addition to the formulations described previously, a preparation of the present invention may also be formulated for local administration, such as a depot preparation. Such long acting formulations may be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the preparation may be formulated with suitable polymeric or hydrophobic materials (for example, as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives such as sparingly soluble salts. Formulations for topical administration may include, but are not limited to, lotions, suspensions, ointments gels, creams, drops, liquids, sprays emulsions and powders.

[0125] According to a preferred embodiment of the present invention, the pharmaceutical composition is designed for a slow release of the tyrphostin preparation. The composition includes particles including a slow release carrier (typically, a polymeric carrier), such as, for example, polylactic acid, and the tyrphostin preparation. Slow release biodegradable carriers are well known in the art. These are materials that may form particles that may capture therein an active compound(s) and slowly degrade/dissolve under a suitable environment (e.g., aqueous, acidic, basic, etc.) and thereby degrade/dissolve in body fluids and release the active compound(s) therein. The particles are preferably nanoparticles (i.e., in the nanometer range, e.g., in the range of about 1 to about 500 nm in diameter, preferably about 50-200 nm in diameter, most preferably about 100 nm in diameter).

[0126] Further according to the present invention there is provided a method of preparing a pharmaceutical composition for slow release of a tyrphostin.

[0127] The method includes the following steps:

First, an isomer-enriched tyrphostin preparation is provided, comprising of the above-described compounds.

Second, a slow release carrier (typically, a polymeric carrier) and the isomer-enriched tyrphostin preparation are dissolved or dispersed in an organic solvent for obtaining an organic solution containing the carrier and the isomer-enriched tyrphostin preparation;

Third, the organic solution is added into an aqueous solution for obtaining an oil-in-water-type emulsion. Preferably,

the aqueous solution includes surface-active agent(s).
Fourth, the organic solvent is evaporated from the oil-in-water-type emulsion for obtaining a colloidal suspension of particles containing the slow release carrier and the isomer-enriched tyrphostin preparation.

[0128] According to a preferred embodiment of the present invention the slow release carrier is polylactic acid.

[0129] Further according to the present invention there is provided a stent, comprising a substantially tubular body, the body is made of or coated with a material designed for slow release of a tyrphostin preparation as described herein

[0130] Specifically, a slow release formulation of the tyrphostin preparation can be used in patients undergoing balloon angioplasty, stent deployment, coronary artery bypass surgery, and heart transplantation as a preventive process of restenosis (see below, "The biochemistry").

[0131] The pharmaceutical compositions herein described may also comprise suitable solid of gel phase carriers or excipients. Examples of such carriers or excipients include, but are not limited to, calcium carbonate, calcium phosphate, various sugars, starches, cellulose derivatives, gelatin and polymers such as polyethylene glycols.

[0132] Many of the PTK modulating compounds in the claimed preparations of the present invention may be provided as physiologically acceptable salts wherein the compound may form the negatively or the positively charged species. Examples of salts in which the compound forms the positively charged moiety include, without limitation, quaternary ammonium (defined elsewhere herein), salts such as the hydrochloride, sulfate, carbonate, lactate, tartrate, maleate, succinate, etc, wherein the nitrogen of the quaternary ammonium group is a nitrogen of a compound of the present invention which reacts with an appropriate acid. Salts in which the compound forms the negatively charged species include, without limitation, the sodium, potassium, calcium and magnesium salts formed by the reaction of a carboxylic acid group in the molecule with the appropriate base (e.g., sodium hydroxide (NaOH), potassium hydroxide (KOH), calcium hydroxide ($Ca(OH)_2$), etc.).

[0133] ***Dosage:*** Pharmaceutical compositions suitable for use in context of the present invention include compositions wherein the active ingredients are contained in an amount effective to achieve the intended purpose. More specifically, a therapeutically effective amount means an amount of tyrphostin preparation effective to prevent, alleviate or ameliorate symptoms of disease or prolong the survival of the subject being treated.

[0134] Determination of a therapeutically effective amount is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein.

[0135] For any preparation used in the methods of the invention, the therapeutically effective amount or dose can be estimated initially from cell culture assays. For example, a dose can be formulated in animal models to achieve a circulating concentration range that includes the IC_{50} as determined in cell culture (i.e., the concentration of the test compound, which achieves a half-maximal inhibition of the PTK activity). Such information can be used to more accurately determine useful doses in humans.

[0136] Toxicity and therapeutic efficacy of the compounds described herein can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., by determining the IC_{50} and the LD_{50} (lethal dose causing death in 50 % of the tested animals) for a subject compound. The data obtained from these cell culture assays and animal studies can be used in formulating a range of dosage for use in human. The dosage may vary depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition. (See e.g., Fingl, et al., 1975, in "The Pharmacological Basis of Therapeutics", Ch. 1 p.1).

[0137] Dosage amount and interval may be adjusted individually to provide plasma levels of the active moiety which are sufficient to maintain the kinase modulating effects, termed the minimal effective concentration (MEC). The MEC will vary for each preparation, but can be estimated from *in vitro* data; e.g., the concentration necessary to achieve 50-90 % inhibition of a kinase may be ascertained using the assays described herein. Dosages necessary to achieve the MEC will depend on individual characteristics and route of administration. HPLC assays or bioassays can be used to determine plasma concentrations.

[0138] Dosage intervals can also be determined using the MEC value. Preparations should be administered using a regimen, which maintains plasma levels above the MEC for 10-90 % of the time, preferable between 30-90 % and most preferably 50-90 %.

[0139] It is noted that, in the case of local administration or selective uptake, the effective local concentration of the drug may not be related to plasma concentration. In such cases, other procedures known in the art can be employed to determine the effective local concentration.

[0140] Depending on the severity and responsiveness of the condition to be treated, dosing can also be a single administration of a slow release composition described hereinabove, with course of treatment lasting from several days to several weeks or until cure is effected or diminution of the disease state is achieved.

[0141] The amount of a composition to be administered will, of course, be dependent on the subject being treated, the severity of the affliction, the manner of administration, the judgment of the prescribing physician, etc.

[0142] ***Packaging:*** Compositions of the present invention may, if desired, be presented in a pack or dispenser device,

such as an FDA approved kit, which may contain one or more unit dosage forms containing the active ingredient. The pack may, for example, comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration. The pack or dispenser may also be accompanied by a notice associated with the container in a form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals, which notice is reflective of approval by the agency of the form of the compositions or human or veterinary administration. Such notice, for example, may be of labeling approved by the U.S. Food and Drug Administration for prescription drugs or of an approved product insert. Compositions comprising a preparation of the invention formulated in a compatible pharmaceutical carrier may also be prepared, placed in an appropriate container, and labeled for treatment of an indicated condition. Suitable conditions indicated on the label may include treatment of a tumor, inhibition of angiogenesis, treatment of fibrosis, diabetes and the like.

The biochemistry:

[0143] In yet another embodiment, the present invention relates to a method for the modulation of the catalytic activity of PTKs. The method is effected by administering a preparation of the present invention or a physiologically acceptable salt or prodrug thereof to a PTK.

[0144] By "PTK" is meant both receptor tyrosine kinases (RTKs) and cellular tyrosine kinases (CTKs or non-receptor TKs); i.e., the modulation of both RTK signal transduction and CTK signal transduction is contemplated by the present invention.

[0145] The term "method" refers to manners, means, techniques and procedures for accomplishing a given task including, but not limited to, those manners, means, techniques and procedures either known to, or readily developed from known manners, means, techniques and procedures by practitioners of the chemical, pharmacological, biological, biochemical and medical arts.

[0146] As used herein, the term "modulation" refers to the alteration of the catalytic activity of RTKs and/or CTKs. In particular, modulation refers to the inhibition of the catalytic activity of RTKs and/or CTKs.

[0147] The term "catalytic activity" as used herein refers to the rate of phosphorylation of tyrosine under the influence, direct or indirect, of RTKs and/or CTKs.

[0148] The term "administering" as used herein refers to a method for bringing a tyrphostin preparation of the present invention and a target PK together in such a manner that the tyrphostin can affect the catalytic activity of the PK either directly; i.e., by interacting with the kinase itself or indirectly; i.e., by interacting with another molecule on which the catalytic activity of the kinase is dependent. As used herein, administration can be accomplished either *in vitro*, i.e. in a test tube, or *in vivo*, i.e., in cells or tissues of a living organism (see below).

[0149] A precise understanding of the mechanism by which the tyrphostin preparations of the present invention inhibits PTKs is not required in order to practice the present invention, however, while not being bound to any particular mechanism or theory, it is believed that tyrphostins interact with the amino acids of the catalytic region of PTKs. PTK typically possess a bi-lobate structure wherein ATP appears to bind in the cleft between the two lobes in a region where the amino acids are conserved among PTKs. Inhibitors of PTKs are believed to bind by non-covalent interactions such as hydrogen bonding, Van der Waals forces and ionic interactions in the same general region where the aforesaid ATP binds in the general space normally occupied by the adenine ring of ATP, i.e., PTK inhibitors are suggested to act as biomimetics to the ATP molecule. More specifically, it is thought that the quinoxaline ring component of tyrphostins binds in the general space normally occupied by the adenine ring of ATP. Recent studies have suggested that the profound selective PTK inhibition of such compounds results from competitive or mixed competitive interaction with the ATP binding domain as well as mixed competitive inhibition with substrate binding sub-sites [23]. Specificity of a particular quinoxaline for a particular PTK may arise as the result of additional interactions between the various substituents on the quinoxaline core and the amino acid domain specific to particular PTKs. Thus, the geometrical tyrphostin isomers of the present invention are formed during the synthetic procedure, which may have inherent differential capability to bind at the ATP binding domain, and hence selectivity towards certain PTK, such as PDGFR, and additional differential potencies at the specific PTK, e.g., PDGFR.

[0150] Further according to the present invention there is provided a method of inhibiting cell proliferation by subjecting the cells to a tyrphostin preparation of the compounds described hereinabove. In a preferred embodiment of the invention the cells are of an organism (e.g., a human), whereas subjecting the cells to the tyrphostin compound is effected *in vivo*. Alternatively, subjecting the cells to the tyrphostin compound is effected *in vitro*.

[0151] Thus, further according to the present invention there is provided a method of treating or preventing a protein tyrosine kinase related disorder or disease of an organism, such as a mammal (e.g., a human) by administering a therapeutically effective amount of the pharmaceutical composition as described above to the organism.

[0152] Herein, the term "treating" includes abrogating, substantially inhibiting, slowing or reversing the progression of a disease, substantially ameliorating clinical symptoms of a disease or substantially preventing the appearance of clinical symptoms of a disease.

[0153] Herein, the term "preventing" refers to a method for barring an organism from acquiring a disorder or disease in the first place.

[0154] As used herein, "PTK related disorder" refers to a disorder characterized by inappropriate PTK activity or over-activity of the PTK. Inappropriate activity refers to either; (i) PTK expression in cells which normally do not express PTKs; (ii) increased PTK expression leading to unwanted cell proliferation, differentiation and/or growth; or, (iii) decreased PTK expression leading to unwanted reductions in cell proliferation, differentiation and/or growth. Over-activity of PTKs refers to either amplification of the gene encoding a particular PTK or production of a level of PTK activity which can correlate with a cell proliferation, differentiation and/or growth disorder (that is, as the level of the PTK increases, the severity of one or more of the symptoms of the cellular disorder increases). Over activity can also be the result of ligand independent or constitutive activation as a result of mutations such as deletions of a fragment of a PTK responsible for ligand binding.

[0155] Thus, the PTK mediated disorders which are the object of the present invention can be studied, prevented or treated by the methods set forth herein whether the cells or tissues of the organism exist within the organism or outside the organism. Cells existing outside the organism can be maintained or grown in cell culture dishes. In this context, the ability of a particular compound to affect a PTK related disorder can be determined, e.g., the IC_{50} of the compound can be ascertained before the use of the compounds in more complex living organisms is attempted. For cells outside the organism, multiple methods exist, and are well known to those skilled in the art, to administer compounds including, but not limited to, direct cell microinjection and numerous transmembrane carrier techniques. For cells harbored within a living organism, myriad methods also exist, and are likewise well-known to those skilled in the art, to administer compounds including, but not limited to, oral, parenteral, dermal and aerosol applications.

[0156] The term "organism" refers to any living entity comprised of at least one cell. A living organism can be as simple as, for example, a single eukaryotic cell or as complex as a mammal, including a human being.

[0157] The term "therapeutically effective amount" refers to that amount of the compound being administered which will relieve to some extent one or more of the symptoms of the disorder being treated.

[0158] The present invention is thus directed to tyrphostins-containing-preparations, which modulate PTK activity signal transduction by affecting the enzymatic activity of the RTKs and CTKs and thereby interfering with the signal transduced, by such proteins.

[0159] Examples, without limitation, of the types of disorders related to unregulated PTK activity that the preparations described herein may be useful in preventing, treating and studying are fibrotic disorders, metabolic disorders and cell proliferative disorders, related to PTKs such as PDGF, EGF, IGF and met.

[0160] Fibrotic disorders refer to the abnormal formation of extracellular matrices. Examples of fibrotic disorders include hepatic cirrhosis and mesangial cell proliferative disorders. Hepatic cirrhosis is characterized by the increase in extracellular matrix constituents resulting in the formation of a hepatic scar. Mesangial cell proliferative disorders refer to disorders brought about by abnormal proliferation of mesangial cells. Mesangial proliferative disorders include various human renal diseases, such as glomerulonephritis, diabetic nephropathy, malignant nephrosclerosis, thrombic microangiopathy syndromes, transplant rejection and glomerulopathies. In this regard, PDGFR has been implicated in the maintenance of mesangial cell proliferation. Other fibrotic disorders implicated include atherosclerosis.

[0161] The association between abnormal PTK activity and disease includes also metabolic diseases, such as psoriasis, diabetes mellitus, wound healing, inflammation and neurodegenerative diseases. For example, EGFR has been indicated in corneal and dermal wound healing. Defects in the Insulin-R and IGF-1R receptor are indicated in type-II diabetes mellitus.

[0162] Cell proliferative disorders which may be prevented, treated or further studied by the present invention include cancer, blood vessel proliferative disorders and mesangial cell proliferative disorders.

[0163] As used herein, the term "cancer" refers to various types of malignant neoplasms, most of which can invade surrounding tissues, and may metastasize to different sites, as defined by Stedman's medical Dictionary 25th edition (Hensyl ed., 1990). Examples of cancers which may be treated by the tyrphostins of the present invention include, but are not limited to, brain, ovarian, colon, prostate, kidney, bladder, breast, lung, oral and skin cancers which exhibit inappropriate PTK activity. These cancers can be further broken down. For example, brain cancers include glioblastoma multiforme, anaplastic astrocytoma, astrocytoma, ependyoma, oligodendroglioma, medulloblastoma, meningioma, sarcoma, hemangioblastoma, and pineal parenchymal. Likewise, skin cancers include melanoma and Kaposi's sarcoma. PTKs have been associated with the development of cancer. Some of the above mentioned PTK receptors, like EGFR and PDGFR, are over-expressed in many tumors and/or are persistently activated by autocrine loops have been demonstrated [31-33]. Specifically, PDGFR has been associated with glioblastoma, melanoma and lung, ovarian, and prostate cancer.

[0164] Blood vessel proliferative disorders refer to angiogenic and vasculogenic disorders generally resulting in abnormal proliferation of blood vessels. The formation and spreading of blood vessels, or vasculogenesis and angiogenesis, respectively, play important roles in a variety of physiological processes such as embryonic development, corpus luteum formation, wound healing and organ regeneration. They also play a pivotal role in cancer development. Other

examples of blood vessel proliferation disorders include arthritis, where new capillary blood vessels invade the joint and destroy cartilage, and ocular diseases, like diabetic retinopathy, where new capillaries in the retina invade the vitreous, bleed and cause blindness. Conversely, disorders related to the shrinkage, contraction or closing of blood vessels, such as restenosis, are also implicated. Of special relevance to the above described proliferative disorders are proliferation and migration processes involving activated smooth muscle cells (SMC), which are associated with release of abundant extracellular matrix by these cells, and are fundamental to neointimal growth associated with accelerated arteriosclerosis which continues to plague patients undergoing balloon angioplasty, stent deployment, coronary artery bypass surgery, and heart transplantation. Injury to the vessel wall, with or without loss or damage to the endothelium, causes a subpopulation of the quiescent, differentiated SMC to lose their contractile myofilamentary apparatus and transform into synthetic cells with large amounts of rough endoplasmic reticulum, ribosomes, and mitochondria. This transformation, directed, at least partially, by PDGF, is associated with SMC migration and proliferation followed by elaboration of abundant extracellular matrix. A variety of experimental studies have been directed toward the attenuation of SMC *in vitro* and *in vivo.* Nonetheless, relatively little progress has been made in the development of effective, selective, non-toxic inhibitors of SMC growth which might eventually be applied in the interventional setting. Recent progress in determining the mechanisms by which growth factors control cell proliferation has contributed to the development of treatment strategies which target specific signal transduction pathways in order to control proliferative disorders.

[0165] Specifically, inhibitors of protein tyrosine kinases (PTKs) have been shown to suppress SMC chemotaxis and proliferation. The tyrphostin phosphorylation inhibitors, which are at the center of the present invention, are low molecular weight, synthetic compounds whose basic structure can be modified to block specific receptor PTKs or intracellular PTKs. Unlike larger receptor antibodies, the small size of the tyrphostins permits easier access to receptor sites within tissues such as in the depths of the media. The development of this class of compounds was based on the concept that it would lead to a more focused control of proliferative disorders, achieve more improved therapeutic indices, and reduce the numerous untoward side effects of the more generalized inhibitors of DNA or RNA synthesis or cytoskeleton-disrupting agents. Indeed, it was recently shown that controlled local delivery of the non-selective PTK blocker AG 17 (RG50872) effectively inhibits neointimal formation in a rat carotid balloon injury model [24].

[0166] The signal transduction induced by PDGF-BB, considered by many to be the strongest known mitogen and chemoattractant for arterial SMC, stimulates directed migration and proliferation of arterial SMC into the neointima following arterial injury. Platelet-derived growth factor (PDGF), expressed by platelets, SMC, endothelial cells, and macrophages, has been shown to play an important role in the pathogenesis of injury-induced neointimal formation in the arterial wall acting as both a mitogen and chemoattractant for SMC as well as being involved in the transformation of SMC from their contractile to the proliferative phenotype. *In vivo* studies have demonstrated that the expression of PDGF ligand and its receptor are elevated following arterial injury.

[0167] Infusion of PDGF into injured rat carotid arteries, or transfection of a plasmid coding for PDGF into porcine arteries, have also been shown to increase neointimal formation. PDGF receptor levels in SMC from human atherosclerotic plaques have also been reported to be elevated compared to receptor levels in normal medial SMC. Recently, *Sirois et al.* [25] have shown marked upregulation of PDGF receptors following injury to the vessel wall. They have demonstrated that the degree of neointimal formation substantially depends on both PDGFR-β overexpression and its activation by PDGF-BB. They demonstrated further that controlled local delivery of antisense oligonucleotides to PDGF-β receptor reduces neointimal formation in the rat carotid injury model. Finally, PTK blockers of the tyrphostin family have been shown to block PDGF receptor signal transduction, including the phosphorylation and activation of PLCγ, believed to be involved in SMC migration [20, 21, 22, 26].

[0168] Thus, further according to the present invention there is provided a method of locally treating a proliferative disorder of a tissue (e.g., an artery) of an organism (e.g., human) by applying a slow release pharmaceutical composition as described above onto the tissue. The disorder may be of any type above mentioned. More specifically, a disorder may be a proliferative disorder, associated with excessive or uncontrolled cell proliferation, including, but not limited to, psoriasis, papilloma, restenosis, atherosclerosis, in-stent stenosis, vascular graft restenosis, pulmonary fibrosis, glomerular nephritis, rheumatoid arthritis and PDGF receptor associated malignancies.

[0169] Additional objects, advantages, and novel features of the present invention will become apparent to one ordinarily skilled in the art upon examination of the following examples, which are not intended to be limiting. Additionally, each of the various embodiments and aspects of the present invention as delineated hereinabove and as claimed in the claims section below finds experimental support in the following examples.

EXAMPLES

[0170] Reference is now made to the following examples, which together with the above descriptions, illustrate the invention in a non limiting fashion. The following protocols and experimental details are referenced in the Examples that follow:

Chemical synthesis of tyrphostins, their analysis and structure verification:

[0171] ***Synthesis of 1,2-dimethyl 5,6-diamino benzimidazole:*** This compound served as the starting material to the synthesis of all tyrphostins compounds as further detailed hereinunder. Synthesis of 1,2-dimethyl 5,6-diamino benzimidazole included the following synthesis steps:

[0172] ***Synthesis of 2-methyl benzimidazole:*** Phenylene diamine (32 grams) and glacial acetic acid (60 ml) were refluxed for 2 hours. Ice and KOH were added to bring the pH to 8.0, and the resulting light violet solid was filtered and collected. Recrystallization from benzene yielded 30 grams of 2-methyl benzimidazole as a light yellow solid having a melting point of 170 °C and total yield of 77 %.

[0173] ***Synthesis of 1,2-dimethyl benzimidazole***: To 10 grams of 2-methyl benzimidazole (75 mM), crushed 25 grams of crushed KOH (450 mM) in 300 ml acetone, methyl iodide (15 grams, 105 mM) was added at a continuous drip for 0.5 hours, at room temperature. Following additional 0.5 hour, water was added, the reaction extracted with dichloromethane, evaporated and chromatographed on silica gel to yield 6 grams (54 %) of 1,2-dimethyl benzimidazole as a white solid having a melting point of 102 °C.

[0174] ***Synthesis of 1,2-dimethyl 5,6-dinitro benzimidazole:***

1,2-dimethyl benzimidazole (3.3 grams) in 15 ml HNO_3 (70 %) was cooled with ice and 10 ml concentrated sulfuric acid was slowly added thereto. The reaction was then stirred at 100 °C for 2 hours, poured on ice and neutralized with KOH. Filtering resulted in 4 grams ,93% yield, of pale blue-white solid. The solid consisted of about 80 % 1:1 mixture of 5-nitro:6- nitro; about 10% 4-nitro; and about 10 % 5,6-dinitro isomers.

[0175] The above mixture (1.5 grams) was treated with 10 ml HNO_3 (70 %) and 6 ml concentrated sulfuric acid at 190 °C for 3.5 hours, poured on ice and neutralized with KOH. Filtering resulted in collection of a light green solid. Recrystallization of that solid from ethanol yielded 0.48 grams (26 % yield) of pure 5,6-dinitro isomer which is a white solid having a melting point of 224 °C, NMR $CDCl_3$ d 8.17(1H,s), 7.90(1H,s), 3.87(3H,s), 2.73 (3H,s).

[0176] ***Synthesis of 1,2-dimethyl 5,6-diamino benzimidazole:***

Pure 1,2-dimethyl 5,6-dinitro benzimidazole (0.7 grams) and 0.2 grams Pd/C in 20 ml ethanol and 20 ml glacial acetic acid were hydrogenated for 4 hours. Filtering and evaporating resulted in 0.5 grams, 95% yield of a white solid having a melting point of 212 °C.

[0177] ***Synthesis of AG2033 and AG2034 (which are geometrical isomers 1851 which is described in*** **US-A-5,932,580**

1,2-dimethyl 5,6-diamino benzimidazole (0.5 grams, 2.8 mM) and 0.45 grams, 3 mM, phenyl glyoxal hydrate in 20 ml ethanol and 20 ml acetic acid were refluxed for 3 hours, neutralized with NaOH, extracted with CH_2Cl_2, evaporated and analytically separated by chromatography on silica gel (TLC). The isomer AG2033 migrates at R_f = 0.6 (5:95 CH_3OH:CH_2Cl_2), while the isomer AG2034 migrates at R_f= 0.5.

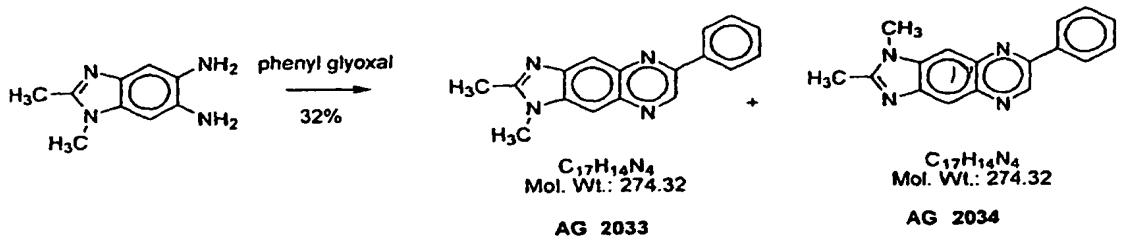
[0178] Preparative separation of the isomers was achieved by chromatography on 150 grams silica gel, 70-230 mesh. Elution was conducted with 1 % methanol in CH_2Cl_2.

(i) AG2033: 1,2-dimethyl-6-phenyl imidazolo[5,4-g]quinoxaline ("transoid"). From first fractions - 0.265 grams (32 % yield), a light yellow solid having a melting point of 275 °C. NMR ($CDCl_3$): 9.30(1H,s,H_7), 8.45(1H,s.H_4), 8.21 (2H,m), 7.95(1H,s,H_9), 7.50(3H,m), 3.88(3H,s,N-methyl), 2.75(3H,s,2-methyl).

(ii) AG2034: 1,2-dimethyl-7-phenyl imidazolo[5,4-g]quinoxaline ("cisoid"). From later fractions - 0.265 grams (32% yield), a light yellow solid having a melting point of 218 °C. NMR ($CDCl_3$): 9.32(1H,s,H_7), 8.42(1H,s.H_4), 8.21(2H, m), 8.0(1H,s,H_9), 7.50(3H,m), 3.88(3H,s,N-methyl), 2.75(3H,s,2-methyl). MS m/e - (AG1851 - mixture of AG2033, AG2034) - 274(M^+, 100%), 259(M- CH_3, 8%), 247(M-HCN, 11%), 144(M-phenyl-HCN-CN, 68%), 129(144-CH_3, 13), 123(15), 102(12), 88(14), 77(15).

[0179] Scheme 1 below illustrates the synthesis and structure of the two isomers, AG2033 and AG2034.

SCHEME 1



[0180] ***Synthesis ofA G2043 and AG2044 (which are geometrical isomers of AG1992 which is described in US-A-5,932,580*** 4.6 grams, 20 mM, 1,2-dimethyl-5,6-dinitro-benzimidazole, hereinabove described, and 0.6 grams 5% Pd/C in 30 ml ethanol and 30 ml acetic acid were hydrogenated for 4 hours. After filtering 6.3 grams, 23 mM, thiophene glyoxal [34] and 1 ml HCl were added and the reaction refluxed for 1.5 hours, neutralized with KOH, extracted with CH_2Cl_2 and evaporated.

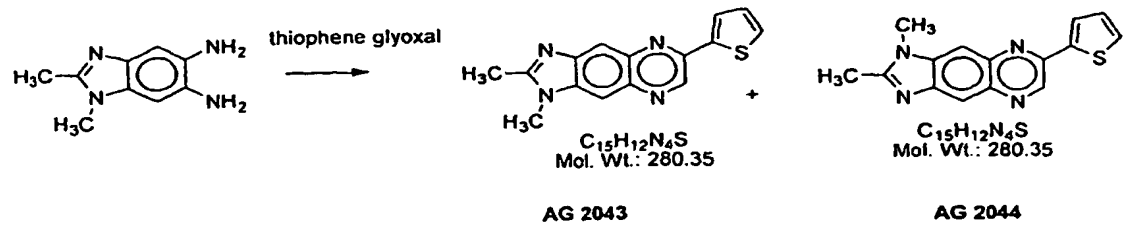
[0181] Preparative separation of the isomers was achieved by chromatography on 200 grams silica gel, 70-230 mesh. Elution was conducted with 1 % methanol in CH_2Cl_2 resulting in:

(i) AG2043: 1,2-dimethyl-6-(2-thiophene) imidazolo[5,4-g]quinoxaline ("transoid"). From first fractions - 0.23 grams (4 % yield), a light yellow solid having a melting point of 274 °C. Rf=0.6 (5:95 CH_3OH:CH_2Cl_2). NMR ($CDCl_3$): 9.20 (1H,s,H_7), 8.34(1H,s.H_4), 7.87(1H,s,H_9), 7.83,7.52,7.20 (3H.ABX 12 line m, thiophene), 3.88 (3H,s,N-methyl), 2.75 (3H,s,2-methyl). NMR (Acetone d_6): 9.38(1H,s,H_7), 8.12(1H,s.H_4), 8.02(1H,s,H_9), 8.08,7.71,7.27 (3H.ABX 12 line m, thiophene), 3.97(3H,s,N-methyl), 2.70 (3H,s,2-methyl). NMR (DMSO d_6): 9.48(1H,s,H_7), 8.15(1H,s.H_4), 8.09 (1H,s,H_9), 8.18,7.81,7.28 (3H.ABX 12 line m, thiophene), 3.88(3H,s,N-methyl), 2.66 (3H,s,2-methyl). NMR (Nitrobenzene d_5): 9.0(1H,s,H_7), 8.01(1H,s.H_4), 7.60(1H,s,H_9), 7.61,7.27, 6.92 (3H.ABX 12 line m, thiophene), 3.50 (3H,s,N- CH_3), 2.38 (3H,s,2- CH_3). MS m/e - (AG1992 - mixture of AG2043, AG2044) - 280 (M ,100%), 253(M-HCN, B%), 144(M-thiophene-HCN-CN,48%), 127(13), 111(11), 88(14), 76(9).

(ii) AG2044: 1,2-dimethyl-7-(2-thiophene) imidazolo[5,4-g]quinoxaline ("cisoid"). From following fractions - 0.6 grams (11% yield), a light yellow solid having a melting point of 218 °C. Rf=0.5 (5:95 CH_3OH:CH_2Cl_2). NMR ($CDCl_3$): 9.22(1H,s,H_6), 8.34(1H,s.H_4), 7.90(1H,s,H_9), 7.83,7.52,7.20 (3H.ABX 12 line m, thiophene), 3.88(3H,s, N-methyl), 2.75(3H,s,2-methyl). NMR (Acetone d_6): 9.38(1H,s,H_7), 8.15(1H,s.H_4), 7.96(1H,s,H_9), 8.08,7.71,7.27 (3H.ABX 12 line m, thiophene), 3.97(3H,s,N-methyl), 2.70(3H,s,2-methyl). NMR (DMSO d_6): 9.46(1H,s,H_7), 8.14 (1H,s.H_4), 8.13(1H,s,H_9), 8.18,7.81,7.28 (3H.ABX 12 line m, thiophene), 3.88(3H,s,N-methyl), 2.66(3H,s,2-methyl). NMR (Nitrobenzene d_5): 9.0(1H,s,H_7), 8.05(1H,s.H_4), 7.47(1H,s,H_9), 7.64,7.32,6.95(3H.ABX 12 line m, thiophene), 3.50(3H,s,N-methyl), 2.38(3H,s,2-methyl).

[0182] Scheme 2 below illustrates the synthesis and structure of the two isomers, AG2043 and AG2044.

SCHEME 2



Cell culture techniques and assays:

Cells and reagents:

[0183] Smooth muscle cells (SMC) were obtained under aseptic conditions from porcine abdominal aortas. Each artery was cut open and the endothelial surface mechanically scraped. The vessels were then cut into 2 mm^2 fragments which were placed in culture dishes with Dulbecco's modified Eagle's medium (DMEM) supplemented with 15 % (v/v) fetal calf serum (FCS), 100 u/ml penicillin, 100 µg/ml streptomycin, and 0.2 M L-glutamine. The tissue fragments were then placed in an incubator at 37 °C under 9 % CO_2 atmosphere until SMC outgrowth was detected (typically within 3-7 days). Uniform populations of SMC which displayed the characteristic "hill and valley" growth pattern were subcultured using 0.25 % trypsin for transfer. For experiments testing the effect of tyrphostins on growth inhibition and recovery (see below), SMC from passages 1 - 3 were replated in 15 mm wells pretreated with 3 µg/cm^2 fibronectin (Biological Industries, Kibbutz Beit Haemek, Israel) at 15,000 cells/well.

[0184] Porcine aortic endothelial cells (PAEC), stably transfected with either PDGF-receptor (PAEC/PDGFβR cells, kindly provided by Dr. L. Claesson-Welsh, Uppsala, Sweden) or VEGF-receptor KDR (PAEC/KDR), respectively, were used as previously described [27, 29]. Cells were routinely cultured in Ham's F-12 medium supplemented with geneticin (0.4 mg/ml) and 10 % FCS.

[0185] Human coronary artery endothelial cells (HCAEC) and human coronary artery smooth muscle cells (HCASMC) were purchased from Clonetics (Heidelberg), and grown in EBM medium (supplemented with EGM-MV SINGLE QUOTS®) or in SmBM medium (supplemented with SmGM-2 SINGLE QUOTS®), respectively (Clonetics).

[0186] All cell culture reagents were from Gibco BRL, unless otherwise indicated. PDGF was the recombinant human BB homodimer. VEGF was obtained from Sigma. Anti-PDGF receptor antiserum DIG-1 was raised against a peptide corresponding to amino acid residues 1075-1089 in the human PDGF α-receptor which recognizes PDGF α- and β-receptors equally well [22]. Antiserum PDGF-R3 against PDGF receptor has been described [5]. [^{32}P]ATP was purchased from DuPont/N-EN (Dreieich, Germany). The polyclonal anti-KDR receptor antiserum NEF was raised against a synthetic peptide as previously described [28]. Additional reagents employed in specific experiments and their sources are indicated below.

[0187] ***In vitro effect of tyrphostins on purified PDGFR autophosphroylation:*** Membranes were prepared from confluent cultures of canine kidney epithelial cells (TRMP) or Swiss 3T3 cells as described [23]. Further purification of the PDGF-βR was performed as described in [23]. Briefly, 2 µl kinase preparation were incubated for 20 minutes on ice in the presence of 2 µg/ml PDGF-BB in 20 µl of 50 mM Hepes (pH 7.4). Kinase reaction was executed for 10 minute on ice with 5 mM $MnCl_2$, 1 mM vanadate, 10 µM [γ-^{32}P]ATP (2.5 µCi per reaction). The reaction was terminated by addition of 10 µl of a solution containing 6 % SDS, 30 % β-mercaptoethanol, 40 % glycerol, and 0.5 mg/ml bromophenol blue. The samples were heated for 5 minutes at 95 °C and subjected to polyacrylamide gel electrophoresis in the presence of 0.4 % SDS, using 7.5 % polyacrylamide gels. The gels were stained, dried and subjected to autoradiographic analysis. For quantification of radioactivity in electrophoresis gels, a Phospho-Imager (Molecular Dynamics, Fuji, or Bio-Rad) was used according to the instructions of the manufacturer. To obtain autoradiograms, objects were exposed to X-ray film (Fuji RX or Kodak X-OMAT) with intensifying screens at -70 °C.

[0188] ***Effect of tyrphostins on PDGF-induced PDGFR autophosphorylation in intact PAEC cells:*** PAEC cells, transfected with either PDGFR or KDR, cultivated in Ham's F-12 medium supplemented with 10 % fetal calf serum (FCS), were synchronized for 20 hours in a medium containing 0.01 % BSA. Following preincubation with AG1295 (which served as a non-isomerable control, see reference 23), AG2033, AG2034, AG2043 or AG2044 for 15 minutes, and with Na_3VO_4 (100 µM) for 5 minutes, the cells were stimulated with PDGF-BB (50 ng/ml) or VEGF-A (50 ng/ml) for 10 minutes at 37 °C. After stimulation, the cells were solubilized in Nonidet P-40 (1 %) containing lysis buffer.

[0189] The analysis of PDGF β-receptor phosphorylation was performed as follows. Cell lysates were subjected to immunoprecipitation using the PDGF β-receptor specific antiserum R3 [5]. The precipitates were subjected to polyacrylamide (7.5 %) gel electrophoresis in presence of sodium dodecyl sulfate (SDS-PAGE) and were thereafter blotted onto a nitrocellulose membrane (Hybond C-EXTRA, Amersham). Phosphorylated proteins were detected by immunoblotting using the horseradish-peroxidase conjugated phosphotyrosine antibodies PY20 and 4G10 (Upstate Biotechnology), followed by application of secondary horseradish-peroxidase conjugated goat-anti-mouse antibody and chemoluminescence-based detection (ECL, Amersham) and autoradiography.

[0190] The analysis of KDR receptor phosphorylation was performed as follows. Cell lysates were subjected to immunoprecipitation using the KDR specific antiserum [27]. The precipitates were subjected to polyacrylamide (7.5 %) gel electrophoresis in presence of sodium dodecyl sulfate (SDS-PAGE) and were thereafter blotted onto a nitrocellulose membrane (Hybond C-EXTRA, Amersham). Phosphorylated proteins were detected by immunoblotting using the horseradish-peroxidase conjugated phosphotyrosine antibody RC20H (Transduction Laboratories), followed by chemoluminescence-based detection (ECL, Amersham) and autoradiography.

[0191] Detection of receptor proteins was performed as follows. Cell lysates were subjected to immunoprecipitation

using the PDGF β-receptor specific antiserum R3 or the KDR specific antiserum, as described above, and the precipitates washed three times and thereafter subjected to SDS-PAGE (7.5 %) and blotting onto a nitrocellulose membrane (Hybond C-EXTRA, Amersham). Receptor proteins were detected by immunoblotting using the horseradish-peroxidase conjugated donkey anti-rabbit antibody (Amersham), followed by chemoluminescence-based detection (ECL, Amersham) and autoradiography.

[0192] ***Inhibition of cell proliferation and recovery:*** Monolayer cell growth inhibition and recovery experiments were repeated 3 or 4 times. Each experiment was performed in triplicate. Approximately 15,000 cells (SMC or PAEC) in 1 ml of culture medium supplemented with either 15 % FCS (SMC) or 10 % FCS (PAEC) were seeded on day 0 in 15 mm-wells precoated with fibronectin (SMC) or uncoated (PAEC). Cultures were treated with 10 μM of the following tyrphostin compounds: AG2033, AG2034, AG2043 and AG2044 (for SMC) or AG2033 (for PAEC) dissolved in 0.1 % DMSO on days 1 and 3. On day 7, cultures were washed and the cells allowed to recover. Typically cells were counted on days 3 and 6 for inhibition, and on day 13 for recovery. The medium supplemented with serum (DMEM for SMC and Ham's F12 for PAEC) was changed every other day throughout the experiment.

[0193] ***Assessment of cell migration (Chemotaxis assay):*** The chemotactic response of HCASMC, PAEC/KDR and PAEC/PDGFR cells was assessed using the modified Boyden chamber (Neuro Probe, Inc.) and collagen-coated polycarbonate filters (Nucleopore) with pore diameters of 8 μm as previously described [27]. Briefly, PAEC/KDR and PAEC/PDGFR cells were cultivated and assayed in Ham's F12 medium containing 10 % FCS. HCASMC were cultivated in SmGM-2 (clonetics) and assayed in SmBM-WM containing 10 % FCS and 0.1 % BSA. To the medium in the lower part of the Boyden chamber, either VEGF or PDGF-BB (10 ng/ml, respectively) were added. AG2033 was added to both upper and lower chamber parts. Suspended cells were given 4 hours for migration, after a preincubation period of 15 minute in AG2033. The number of cells that migrated without PDGF-BB or VEGF stimulation was referred to as 100 % migration (chemokinesis). The assay was performed in triplicate, and five medium-power fields were counted per well using a light microscope (Jenalab).

[0194] ***Assessment of cell proliferation ([^{3}H]thymidine incorporation assay):*** PAEC/KDR and PAEC/PDGFR cells, grown in Ham's F12 medium containing 10 % FCS were seeded sparsely in 12 well culture dishes. After 24 hours, cells were washed two times with Ham's F12 medium containing 1 % FCS and incubated for additional 48 hours with one renewal of medium. Cells were incubated for 15 minute with different concentrations of AG2033 (0.1; 1 and 10 μM) or with the solvent DMSO alone, stimulated with 3 ng/ml VEGF or with 15 ng/ml PDGF-BB for 20 hours, followed by addition of 0.25 μCi of [^{3}H]-thymidine/ml (Amersham) for two hours. High molecular weight [^{3}H]-radioactivity was precipitated using 5% trichloroacetic acid at 4°C for 30 minutes. After two washes with ice-cold H_2O, [^{3}H]-radioactivity was solubilized in 1 M NaOH (400 μl/well) at room temperature for 8 minutes, neutralized by the addition of 2 M HCL (400 μl/well), and quantitated by liquid scintillation counting.

Experimental Results:

EXAMPLE 1

Chemical Analysis

X-ray crystal structure analysis:

AG2043:

[0195] All crystallographic computings were performed using a VAX9000 computer at the Hebrew University of Jerusalem, employing the TEXSAN Structure Analysis Software. Data were acquired using an ENRAF-NONIUS CAD-4 Computer-Controlled Diffractometer. CuK_α (λ =1.54178 Å) radiation with a graphite crystal monochromator in the incident beam was used. The standard CAD-4 centering, indexing, and data collection programs were used. The unit cell dimensions were obtained by a least-squares fit of 20 centered reflections in the range of $23° \leq \theta \leq 27°$.

[0196] Intensity data were collected using the ω-2θ technique to a maximum 2θ of 120°. The scan width, $\Delta\omega$, for each reflection was 0.80±0.15 tanθ. An aperture with a height of 4 mm and a variable width, calculated as 2.0+0.5 tanθ mm, was located 173 mm from the crystal. Reflections were first measured with a scan of 8.24 °/minute. The rate of the final scan was calculated from the preliminary scan results so that the ratio I/σ (I) would be at least 40 but the maximum scan time would not exceed 60 seconds. If in the preliminary scan I/σ (I)<2, this measurement was used as the datum. Scan rates varied from 1.27 to 8.24°/minute. Of the 96 steps in the scan, the first and the last 16 steps were considered to be background. During data collection the intensities of three standard reflections were monitored after every hour of X-ray exposure. No decay was observed. In addition, three orientation standards were checked after 100 reflections to check for the effects of crystal movement. If the standard deviation of the h, k, and 1 values of any orientation reflection exceeded 0.08, a new orientation matrix was calculated on the basis of the recentering of the 20

reference reflections.

[0197] Intensities were corrected for Lorentz and polarization effects. All non-hydrogen atoms were found by using the results of the SHELX-86 direct method analysis (30). After several cycles of refinements, the positions of the hydrogen atoms were calculated, and added to the refinement process. Refinement proceeded to convergence by minimizing the function $\Sigma w(|F_o| - |F_c|)^2$. A final difference Fourier synthesis map showed several peaks less than 0.31 e/Å^3 scattered about the unit cell without a significant feature.

[0198] Table 1 below presents the discrepancy indices, $R=\Sigma| |F_o| - |F_c| |/\Sigma|F_o|$ and $R_w=[\Sigma w(|F_o| - |F_c|)^2/\Sigma w |F\mu_o|^2]^{1/2}$ as well as other pertinent crystallographic data, obtained for the AG2043 pure isomer crystal.

TABLE 1

Crystallographic data for AG2043	
Formula	$C_{15}H_{12}N_4S$
Space group	$P2_1$
a, Å	10.834 (2)
b, Å	19.081 (4)
c, Å	6.618 (1)
β, deg	107.10 (1)
V, Å^3	1307.7 (5)
Z	4
ρcalc., gcm^{-3}	1.42
μ (CuK_α), cm^{-1}	21.46
No. of unique reflections	2007
No. of reflections with $I \geq 3\sigma_1$	1688
R	0.049
R_w	0.062

AG2044:

[0199] All crystallographic computings were performed using a VAX9000 computer at the Hebrew University of Jerusalem, employing the TEXSAN Structure Analysis Software. Data were acquired using an ENRAF-NONIUS CAD-4 Computer-Controlled Diffractometer. CuK_α (λ=1.54178 Å) radiation with a graphite crystal monochromator in the incident beam was used. The standard CAD-4 centering, indexing, and data collection programs were used. The unit cell dimensions were obtained by a least-squares fit of 24 centered reflections in the range of $25° \leq \theta \leq 30°$.

[0200] Intensity data were collected using the ω-2θ technique to a maximum 2θ of 120°. The scan width, Δω, for each reflection was 0.80±0.15tanθ. An aperture with a height of 4 mm and a variable width, calculated as 2.0+0.5 tanθ mm, was located 173 mm from the crystal. Reflections were first measured with a scan of 8.24 °/minute. The rate of the final scan was calculated from the preliminary scan results so that the ratio I/σ (I) would be at least 40 but the maximum scan time would not exceed 60 seconds. If in the preliminary scan I//σ (I)<2, this measurement was used as the datum. Scan rates varied from 1.27 to 8.24 °/minute. Of the 96 steps in the scan, the first and the last 16 steps were considered to be background. During data collection the intensities of three standard reflections were monitored after every hour of X-ray exposure. No decay was observed. In addition, three orientation standards were checked after 100 reflections to check the effects of crystal movement. If the standard deviation of the h, k, and l values of any orientation reflection exceeded 0.08, a new orientation matrix was calculated on the basis of the recentering of the 24 reference reflections.

[0201] Intensities were corrected for Lorentz and polarization effects. All non-hydrogen atoms were found by using the results of the SHELX-86 direct method analysis (30). After several cycles of refinements the positions of the hydrogen atoms were calculated, and added to the refinement process. Refinement proceeded to convergence by minimizing the function $\Sigma w(|F_o| - |F_c|)^2$. A final difference Fourier synthesis map showed several peaks less than 0.37 e/Å^3 scattered about the unit cell without a significant feature.

[0202] Table 2 below presents the discrepancy indices, $R=\Sigma||F_o|-|F_c||/\Sigma|F_o|$ and $R_w=[\Sigma w(|F_o|-|F_c|)^2/\Sigma wF_o|^2]^{1/2}$, as well as other pertinent crystallographic data, obtained for the AG2044 pure isomer crystal.

TABLE 2

Crystallographic data for AG2044	
Formula	$C_{15}H_{12}N_4S$ 1.5H_2O
Space group	$P2_1/c$
a, Å	7.261 (3)
b, Å	17.789 (3)
c, Å	23.293 (4)
β, deg	98.00 (3)
V, $Å^3$	2979 (1)
Z	8
pcalc., gcm^{-3}	1.37
p (CuK_α), cm^{-1}	20.07
No. of unique reflections	4560
No. of reflections with $I \geq 3\sigma_1$	3621
R	0.051
R_w	0.074

[0203] Crystallization of AG2043 and AG2044 from acetonitrile gave single crystals, whose structures were unequivocally determined by X-ray analysis. The unit cell of AG2043 and AG2044 contained two different orientations of each molecule (as shown in the unit cells presented in Figures 1 and 3), with water molecule in the unit cell of AG2044.

[0204] Tables 3 and 4 below present further data characterizing the crystal structure of AG2043, providing intramolecular bond distances (Table 3) and angles parameters (Table 4) involving the nonhydrogen atoms. Tables 5, 6, 7 below present further data characterizing the crystal structure of AG2044, providing intramolecular bond distances (Table 5), intramolecular angles (6) and intermolecular distances involving the nonhydrogen atoms (Table 7).

[0205] Figures 1, 2a-b present the unit cell crystal structure of AG2043, as obtained following crystallization from acetonitrile by X-ray analysis (Figure 1), as well as the molecular structure of AG2043 (Figure 2a-b).

[0206] Figures 3, 4a-b present the unit cell crystal structure of AG2044, as obtained following crystallization from acetonitrile by X-ray analysis (Figure 3), as well as the molecular structure of AG2044 (Figure 4a-b).

[0207] Molecular structure of the presented compounds may thus be divided according to their geometrical arrangement: similar to substituents on double bonded carbons, the substituents can either reside on the same side of the double bond (cis), or on opposing sides (trans). Thus, substituents on the nitrogens of the imidazole ring (terminal 5-member ring) at positions 1 and 2 can either reside on the same side as the aryl substituent in the terminal 6 member ring (position 7 of the compound), forming a "cis-like" geometrical arrangement ("cisoid"), or on opposing side (position 6 of the compound), forming a "trans-like" geometrical arrangement ("transoid").

[0208] The data shown herein proves the structure of the more potent isomer, AG2043, (see below, biological activity results), to be the "transoid" structure, i.e., 1,2-dimethyl-6-thiophene (Figure 2a-b), where the 1-Methyl and the thiophene ring are "trans" to each other, and AG2044 the "cisoid" structure, 1,2-dimethyl-7-thiophene analog (Figure 4a-b).

[0209] Turning to evaluate chemical and biological characteristics of the AG2033 and AG2034 isomers pair, similar results were obtained: migration rate on silica gel (TLC) yielded identical R_f's (0.5 and 0.6 for AG2034 and AG2033, respectively) as well as NMR structure analysis. Additionally, evaluation of *in vitro* inhibition of PDGFβR autophosphorylation resulted in differential potencies of the compounds, proving AG2033 to be the more potent isomer compared to AG2034 (see biological results, below). Thus, by analogy to the AG2043 and AG2044 isomers pair, the pair of inhibitors AG2033 and AG2034 (in which a phenyl ring substitutes the thiophene) is assumed to have the "transoid" structure for AG2033.

TABLE 3

Intramolecular distances involving the nonhydrogen atoms A G2043					
atom	**atom**	**distance**	**atom**	**atom**	**distance**
S (1)	C (1)	1.733 (6)	C (1)	C (5)	1.454 (9)
S (1)	C (4)	1.700 (8)	C (2)	C (3)	1.41 (1)
S (2)	C (16)	1.709 (6)	C (3)	C (4)	1.33 (1)

TABLE 3 (continued)

Intramolecular distances involving the nonhydrogen atoms A G2043					
atom	**atom**	**distance**	**atom**	**atom**	**distance**
S (2)	C (19)	1.703 (9)	C (5)	C (6)	1.420 (9)
N (1)	C (5)	1.328 (7)	C (7)	C (8)	1.430 (8)
N (1)	C (8)	1.371 (7)	C (7)	C (12)	1.396 (8)
N (2)	C (6)	1.291 (8)	C (8)	C (9)	1.405 (8)
N (2)	C (7)	1.380 (7)	C (9)	C (10)	1.383 (8)
N (3)	C (10)	1.386 (8)	C (10)	C (11)	1.425(8)
N (3)	C (13)	1.297 (8)	C (11)	C (12)	1.363 (8)
N (4)	C (11)	1.371 (7)	C (13)	C (14)	1.471 (9)
N (4)	C (13)	1.392 (7)	C (16)	C (17)	1.359 (9)
N (4)	C (15)	1.442 (8)	C (16)	C (20)	1.469 (9)
N (5)	C (20)	1.314 (8)	C (17)	C (18)	1.44 (1)
N (5)	C (23)	1.369 (8)	C (18)	C(19)	1.31 (1)
N (6)	C (21)	1.309 (8)	C (20)	C (21)	1.410(9)
N (6)	C (22)	1.367 (7)	C (22)	C (23)	1.447 (8)
N (7)	C (25)	1.397 (8)	C (22)	C (27)	1.386 (8)
N (7)	C (28)	1.306 (8)	C (23)	C (24)	1.383 (8)
N (8)	C (26)	1.378 (8)	C (24)	C (25)	1.375 (8)
N (8)	C (28)	1.376 (8)	C (25)	C (26)	1.417 (8)
N (8)	C (30)	1.461 (8)	C (26)	C (27)	1.372 (8)
C (1)	C (2)	1.362 (9)	C (28)	C (29)	1.48 (1)

[0210] Distances are in angstroms. Estimated standard deviations in the least significant figure are given in parentheses.

TABLE 4

Intramolecular bond angles involving the nonhydrogen atoms AG2043							
atom	**atom**	**atom**	**angle**	**atom**	**atom**	**atom**	**angle**
C (1)	S (1)	C (4)	91.3(4)	C (8)	C (7)	C (12)	121.2 (5)
C (16)	S (2)	C (19)	91.3 (4)	N (1)	C (8)	C (7)	120.8 (5)
C (5)	N (1)	C (8)	117.1 (5)	N (1)	C (8)	C (9)	118.0 (5)
C (6)	N (2)	C (7)	117.0 (5)	C (7)	C (8)	C (9)	121.1 (6)
C (10)	N (3)	C (13)	106.1 (5)	C (8)	C (9)	C (10)	117.1 (5)
C (11)	N (4)	C (13)	107.0 (5)	N (3)	C (10)	C (9)	130.2 (5)
C (11)	N (4)	C (15)	125.8 (5)	N (3)	C (10)	C (11)	109.4 (5)
C (13)	N (4)	C (15)	127.2 (5)	C (9)	C (10)	C (11)	120.4 (5)
C (20)	N (5)	C (23)	117.8 (5)	N (4)	C (11)	C (10)	104.9 (5)
C (21)	N (6)	C (22)	116.9(5)	N (4)	C (11)	C (12)	131.5 (6)
C (25)	N (7)	C (28)	105.0(5)	C (10)	C (11)	C (12)	123.5 (6)
C (26)	N (8)	C (28)	107.1 (5)	C (7)	C (12)	C (11)	116.6 (5)
C (26)	N (8)	C (30)	125.1 (5)	N (3)	C (13)	N (4)	112.6 (5)
C (28)	N (8)	C (30)	127.8 (6)	N (3)	C (13)	C (14)	126.1 (5)
S (1)	C (1)	C (2)	110.3 (5)	N (4)	C (13)	C (14)	121.3 (6)
S (1)	C (1)	C (5)	119.2(4)	S (2)	C (16)	C (17)	111.7 (5)
C (2)	C (1)	C (5)	130.4 (6)	S (2)	C (16)	C (20)	120.2 (5)
C (1)	C (2)	C (3)	112.7 (6)	C (17)	C (16)	C (20)	128.1 (6)
C (2)	C (3)	C (4)	113.1 (7)	C (16)	C (17)	C (18)	111.3 (7)
S (1)	C (4)	C (3)	112.5 (6)	C (17)	C (18)	C (19)	112.6 (8)

TABLE 4 (continued)

Intramolecular bond angles involving the nonhydrogen atoms AG2043							
atom	**atom**	**atom**	**angle**	**atom**	**atom**	**atom**	**angle**
N (1)	C (5)	C (1)	117.6 (5)	S (2)	C (19)	C (18)	113.1 (7)
N (1)	C (5)	C (6)	121.0 (6)	N (5)	C (20)	C (16)	117.4 (5)
C (1)	C (5)	C (6)	121.3 (6)	N (5)	C (20)	C (21)	121.2(6)
N (2)	C (6)	C (5)	123.9 (6)	C (16)	C (20)	C (21)	121.3 (6)
N (2)	C (7)	C (8)	120.1 (5)	N (6)	C (21)	C (20)	123.8 (6)
N (2)	C (7)	C (12)	118.7 (5)	N (6)	C (22)	C (23)	120.0 (5)
N (6)	C (22)	C (27)	119.0 (5)	C (23)	C (22)	C (27)	120.9 (5)
N (5)	C (23)	C (22)	120.1 (5)	N (5)	C (23)	C (24)	119.9 (5)
C (22)	C (23)	C (24)	119.9 (6)	C (23)	C (24)	C (25)	119.3 (6)
N (7)	C (25)	C (24)	130.6 (5)	N (7)	C (25)	C (26)	109.7 (5)
C (24)	C (25)	C (26)	119.7(5)	N (8)	C (26)	C (25)	104.8 (5)
N (8)	C (26)	C (25)	104.8 (5)	N (8)	C (26)	C (27)	132.1 (5)
C (25)	C (26)	C (27)	123.1 (6)	C (22)	C (27)	C (26)	117.1 (5)
N (7)	C (28)	N (8)	113.4 (6)	N (7)	C (28)	C (29)	125.2 (6)
N (8)	C (28)	C (29)	121.4 (6)				

[0211] Angles are in degrees. Estimated standard deviations in the least significant figure are given in parentheses.

TABLE 5

Intramolecular distances involving the nonhydrogen atoms A G2044					
atom	**atom**	**distance**	**atom**	**atom**	**distance**
S (1)	C (1)	1.712 (3)	C (1)	C (5)	1.457 (4)
S (1)	C (4)	1.685 (4)	C (2)	C (3)	1.440 (5)
S (2)	C (16)	1.718 (3)	C (3)	C (4)	1.346 (5)
S (2)	C(19)	1.712 (3)	C (5)	C (6)	1.417 (4)
N (1)	C (5)	1.321 (4)	C (7)	C (8)	1.430 (4)
N (1)	C (8)	1.361 (4)	C (7)	C (12)	1.394 (4)
N (2)	C (6)	1.304 (4)	C (8)	C (9)	1.407 (4)
N (2)	C (7)	1.372 (4)	C (9)	C (10)	1.368 (4)
N (3)	C (10)	1.382 (4)	C (10)	C (11)	1.418 (4)
N (3)	C (13)	1.367 (4)	C (11)	C (12)	1.379 (5)
N (3)	C (14)	1.447 (4)	C (13)	C (15)	1.496 (5)
N (4)	C (11)	1.386 (4)	C (16)	C (17)	1.370 (4)
N (4)	C (13)	1.301 (5)	C (16)	C (20)	1.455 (4)
N (5)	C (20)	1.319 (4)	C (17)	C (18)	1.417 (5)
N (5)	C (23)	1.370 (3)	C (18)	C (19)	1.332 (5)
N (6)	C (21)	1.294 (4)	C (20)	C (21)	1.428 (4)
N (6)	C (22)	1.374 (4)	C (22)	C (23)	1.439 (4)
N (7)	C (25)	1.380 (4)	C (22)	C (27)	1.386 (4)
N (7)	C (29)	1.447 (4)	C (24)	C (25)	1.370(4)
N (8)	C (26)	1.388 (4)	C (25)	C (26)	1.418 (4)
N (8)	C (28)	1.312 (4)	C (26)	C (27)	1.370 (4)
C (1)	C (2)	1.396 (4)	C (28)	C (30)	1.478 (4)

[0212] Distances are in angstroms. Estimated standard deviations in the least significant figure are given in parentheses.

TABLE 6

Intramolecular bond angles involving the nonhydrogen atoms AG2044							
atom	atom	atom	angle	atom	atom	atom	angle
C (1)	S (1)	C (4)	92.3 (2)	C (8)	C (7)	C (12)	120.9 (3)
C (16)	S (2)	C (19)	91.5 (2)	N (1)	C (8)	C (7)	121.2 (3)
C (5)	N (1)	C (8)	117.4 (2)	N (1)	C (8)	C (9)	118.5 (3)
C (6)	N (2)	C (7)	117.1 (3)	C (7)	C (8)	C (9)	120.3 (3)
C (10)	N (3)	C (13)	106.3 (3)	C (8)	C (9)	C (10)	117.3 (3)
C (10)	N (3)	C (14)	125.3 (3)	N (3)	C (10)	C (9)	131.8 (3)
C (13)	N (3)	C (14)	128.2 (3)	N (3)	C (10)	C (11)	105.4 (3)
C (11)	N (4)	C (13)	105.7 (3)	C (9)	C (10)	C (11)	122.8 (3)
C (20)	N (5)	C (23)	117.2 (3)	N (4)	C (11)	C (10)	108.9 (3)
C (21)	N (6)	C (22)	117.2 (3)	N (4)	C (11)	C (12)	130.7 (3)
C (25)	N (7)	C (28)	106.9 (2)	C (10)	C (11)	C (12)	120.4 (3)
C (25)	N (7)	C (29)	125.1 (2)	C (7)	C (12)	C (11)	118.2 (3)
C (28)	N (7)	C (29)	128.0 (3)	N (3)	C (13)	N (4)	113.6 (3)
C (26)	N (8)	C (28)	105.5 (3)	N (3)	C (13)	C (15)	122.0 (4)
S (1)	C (1)	C (2)	112.2 (2)	N (4)	C (13)	C (15)	124.4 (4)
S (1)	C (1)	C (5)	119.5 (2)	S (2)	C (16)	C (17)	110.9 (2)
C (2)	C (1)	C (5)	128.3 (3)	S (2)	C (16)	C (20)	119.8 (2)
C (1)	C (2)	C (3)	109.1 (3)	C (17)	C (16)	C (20)	129.3 (3)
C (2)	C (3)	C (4)	114.1 (3)	C (16)	C (17)	C (18)	112.3 (3)
S (1)	C (4)	C (3)	112.3 (3)	C (17)	C (18)	C (19)	113.0 (3)
N (1)	C (5)	C (1)	117.7 (3)	S (2)	C (19)	C (18)	112.3 (3)
N (1)	C (5)	C (6)	120.9 (3)	N (5)	C (20)	C (16)	117.9 (3)
C (1)	C (5)	C (6)	121.3 (3)	N (5)	C (20)	C (21)	121.2 (3)
N (2)	C (6)	C (5)	123.6 (3)	C (16)	C (20)	C (21)	120.9 (3)
N (2)	C (7)	C (8)	119.8 (3)	N (6)	C (21)	C (20)	123.5 (3)
N (2)	C (7)	C (12)	119.3 (3)	N (6)	C (22)	C (23)	119.9 (3)
N (6)	C (22)	C (27)	119.7 (3)	C (23)	C (22)	C (27)	120.4 (3)
N (5)	C (23)	C (22)	120.8 (3)	N (5)	C (23)	C (24)	118.6 (3)
C (22)	C (23)	C (24)	120.5 (3)	C (23)	C (24)	C (25)	117.2 (3)
N (7)	C (25)	C (24)	132.0 (3)	N (7)	C (25)	C (26)	105.2 (2)
C (24)	C (25)	C (26)	122.9 (3)	N (8)	C (26)	C (25)	109.4 (2)
N (8)	C (26)	C (27)	130.6 (3)	C (25)	C (26)	C (27)	120.1 (3)
C (22)	C (27)	C (26)	118.9 (3)	N (7)	C (28)	N (8)	113.1 (3)
N (7)	C (28)	C (30)	121.9 (3)	N (8)	C (28)	C (30)	125.0 (3)

[0213] Angles are in degrees. Estimated standard deviations in the least significant figure are given in parentheses.

TABLE 7

Intermolecular distances involving the nonhydrogen atoms AG2044			
atom	atom	distance	ADC (*)
O (1w)	O (2w)	2.764 (5)	66703
O (1w)	O (3w)	2.777 (5)	1
O (1w)	N (4)	2.825 (4)	1
O (2w)	O (3w)	2.702 (5)	1
O (2w)	N (8)	2.816 (4)	1

[0214] Contacts out to 3.00 angstroms. Estimated standard deviations in the least significant figure are given in parentheses.

EXAMPLE 2

Biological Analysis

[0215] ***Inhibition of PDGF-induced tyrosine phosphorylation by tyrphostins in vitro:*** A preparation of purified PDGFR from Swiss 3T3 cell membranes was used to assess and compare the inhibitory effects of tyrphostin compounds on tyrosine kinase activity. Various concentrations of the purified "transoid" isomers AG2033 and AG2043, were evaluated. Table 8 below presents IC_{50} values (50 % inhibition of phosphorylation, μ M) of AG2033 and AG2043. Data presents the potencies of the compounds with respect to PDGFR, as was determined using the isolated receptor (see experimental methods section above).

TABLE 8

Concentration (μM)	% of Control AG2033	% of Control AG2043
0	100	100
0.03	97.9	60.1
0.1	37	41.6
0.3	17.9	40.4
1	13.7	15.1
3	9.8	11
IC_{50}	0.07	0.09

[0216] Kinase reactions, using an isolated PDGFβR preparation, in the presence of 0.03-3 μM tyrphostin, were conducted, utilizing [γ-^{32}P]ATP as probe. Analysis was conducted by subjecting samples to SDS-PAGE, radiograms thereof are presented in Figure 5. Densitometric evaluation, relative to control lacking the tyrphostin inhibitor (100%), results in the percent autokinase activity, presented in Table 8 and in the dose-response curves for the purified transoid isomers AG2033 (Figure 6) and AG2043 (Figure 7). Further comparison between the resulting autoradiogrms for each pair of geometrical isomers AG2033 and AG2034 (upper panel); AG2043 and AG2044 (lower panel), for their inhibitory activity towards PDGF-induced PDGFβR autophosphorylation (concentration range: 0.1-10 μM) in intact cells is presented in Figure 8. While marked inhibitory activity is present for the "transoid" isomer AG2033 at 1 μM, similar inhibition exists only at much higher concentrations (10-30 μM) of the "cisoid" isomer AG2034. This data proves AG2033 as having superior activity (higher potency) compared to the AG2034 isomer. Similar potency relation exists between the AG2043 and AG2044 isomers.

Inhibition of PDGF-induced tyrosine phosphorylation of intact cells by tyrphostins:

[0217] Stimulation of porcine aortic endothelial cells (PAEC) with PDGF-BB (100 ng/ml) resulted in strong phosphorylation of the PDGF β-receptor on tyrosine residues. Addition of tyrphostin compounds to the cells prior to PDGF-stimulation completely inhibited PDGF β-receptor tyrosine-phosphorylation. Table 9 below presents IC_{50} values (50 % inhibition of phosphorylation, μM) of various tyrphostins, AG1295 (which serves as a positive control), and the purified isomers of AG1851: AG2033, AG2034 and AG1992: AG2043, AG2044. The data in Table 9 presents the differential potencies of the various compounds with respect to PDGFR and KDR, as was performed on intact PAEC cells expressing these receptors (see experimental methods section above).

TABLE 9

Compound	PDGFR	KDR
AG2033	0.5	>10
AG2034	10.0	n.d.
AG2043	1.0	3.0
AG2044	5.0	n.d.
AG1295	n.d.	1.0
n.d = not determined		

[0218] These compounds do not inhibit other tyrosine kinase receptors at concentrations below 75 μM. The only receptor affected by the above mentioned compounds is the KDR/VEGFR, but only at concentrations that are at least 3-20-fold higher as compared to those effecting PDGFR kinase. Thus, these compounds constitute effective and selective PDGFR kinase blockers. Furthermore, for each isomers' pair (AG2033, AG2034 and AG2043, AG2044) higher potencies are evident for the "transoid" isomer (AG2033, AG2043) relative to the "cisoid" isomer (AG2034, AG2044).

Effects of tyrphostins on cell proliferation:

[0219] ***Pig heart smooth muscle cells (SMC):*** Treatment of pig heart smooth muscle cells (SMC) with AG1992-derived purified isomers, AG2043 and AG2044, as well as AG1295 (10 μM each) resulted in 46 %, 84 % and 61 %, respectively, mean reduction in SMC count by day 3 as is compared to DMSO treated control cells. As further described below, the inhibitory effect of AG2043, AG2044 and AG1295 was completely reversible.

[0220] Figure 9 demonstrates the inhibitory and recovery effects of AG1992-derived purified isomers, AG2043 and AG2044, as well as AG1295 on pig heart SMC proliferation. Cells were grown in the presence of the specified tyrphostins and were counted on days 3, 6 and 13 in culture. On day 7 the cultures were washed and the cells allowed to recover. All three tyrphostins showed potent growth inhibition effect as compared with controls. The "transoid" isomer, AG2043, exhibited higher inhibitory potency as compared its counterpart "cisoid" isomer, AG2044. At the 10 μM concentration, AG2043 induced the most effective inhibition, without having a substantial toxic effect on the cells. The inhibitory effect of all three compounds was reversible, and the cells resumed normal growth response as soon as the treatment with the tyrphostins was withdrawn.

Porcine aortic endothelial cells (PAEC):

[0221] Inhibitory effects of tyrphostin compounds on PDGF-induced proliferation were further evaluated on PAEC cells.

[0222] Table 10 below presents the IC_{50} values (50 % inhibition of proliferation, μM) of purified AG2033 isomer, on PDGFR and KDR of transfected PAEC cells.

Table 10

Compound	PDGFβR	KDR
AG2033	2.5	>10

[0223] Similar to the inhibition results obtained on the respective receptors' autophosphorylation, the purified tyrphostin AG2033 demonstrated potent and selective inhibition of PDGFβR transfected PAEC cell proliferation. Similar results were obtained with human coronary artery endothelial cells (HCAEC).

Effects of tyrphostins on cell migration:

[0224] Evaluation of PDGFβR or KDR stably transfected PAEC cells migration toward the respective growth factors, PDGD-BB and VEGF (10 ng/ml, respectively), in the presence of the purified AG2033 isomer, was conducted in a Boyden chamber (see experimental section, above). Table 11 below presents IC_{50} values (50 % inhibition of migration, μM) for the AG2033 isomer with respect to PDGFR and KDR, as was performed on PAEC cells.

Table 11

Compound	PDGFβR	KDR
AG2033	5.0	>10

[0225] In high accordance with the inhibition of autophosphorylation results described above with respect to PAEC cells, as well as with the proliferation results, the purified tyrphostin AG2033 demonstrates potent and selective inhibition of PAEC cell migration.

[0226] Further evaluation of HCASMC cells migration toward PDGD-BB, in the presence of purified "transoid" isomers, was conducted in a Boyden chamber (see experimental section, above).

[0227] Figure 10 presents data obtained with AG2033. Potent inhibition of the purified tyrphostin isomer is evident in both the absence and presence of PDGF-BB growth factor, i.e., dose-dependent inhibition is achieved of both the basal (IC_{50} < 10 μM) and the induced (IC_{50} < 3 μM) migratory activity of the HCASMC cells. These results correlate with the hereinabove described inhibitory effects of AG2033 on PAEC cell migration, and effects obtained on receptor

autophosphorylation.

[0228] Similarly, Figure 11 presents data obtained with AG2043. Again, potent inhibition of the purified tyrphostin isomer is evident in both the absence and presence of PDGF-BB growth factor, i.e., dose-dependent inhibition is achieved of both the basal (IC_{50} < 10 μM) and the induced (IC_{50} ~ 3 μM) migratory activity of the HCASMC cells. These results correlate with the above described inhibitory effects of AG2043 on receptor autophosphorylation and porcine SMC proliferation.

A pharmaceutical composition of and method for in vitro tyrphostins delivery:

[0229] According to the present invention tyrphostins are delivered to a balloon treated area of an artery by coating the balloon with tyrphostin slow release nanoparticles which slowly discharge the tyrphostin at the balloon treated area, thereby cell proliferation at the treated area is inhibited.

[0230] To this end a tyrphostin compound is formulated in nanoparticles, for example, polylactic acid (PLA) nanoparticles loaded with tyrphostin prepared by an oil-in-water (O/W) emulsification/solvent evaporation method as follows.

[0231] Fifty mg PLA and 3 mg of the selected tyrphostin(s) are dissolved in an organic mixture of 0.5 ml dichloromethane and 10 ml acetone. The organic solution is added to 20 ml of an aqueous solution containing 0.5 % Poloxamer F68. The oil-in-water (O/W)-type emulsion is stirred by means of a magnetic stirrer at 20W power output for 5 minute. The organic solvents are evaporated in a rotating evaporator at pressure of 20 mm Hg, giving a colloidal suspension of nanoparticles. Finally, the obtained suspension is passed through a Whatman 40 filter paper.

[0232] This formulation may be employed for inhibiting cell proliferation via slow release mechanism in various proliferative disorders, including, but not limited to, psoriasis, papilloma, restenosis, atherosclerosis, in-stent stenosis, vascular graft restenosis, pulmonary fibrosis, glomerular nephritis, rheumatoid arthritis and PDGF receptor associated malignancies.

[0233] Although the invention has been described in conjunction with specific embodiments thereof, it is evident that many alternatives, modifications and variations will be apparent to those skilled in the art.

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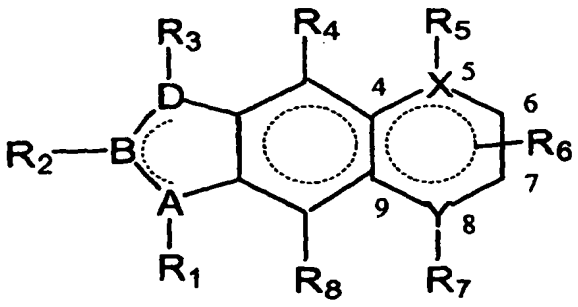
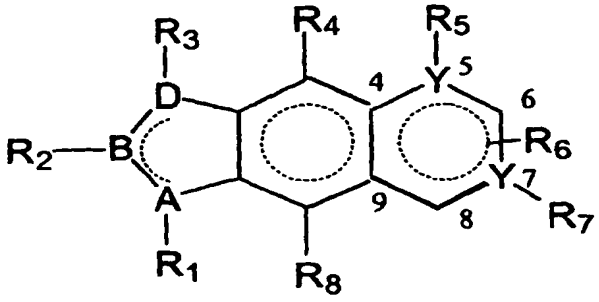
Claims

1. A substantially purified tyrphostin isomer of a general formula:



(Compound I)

or



(Compound II)

wherein

4, 5, 6, 7, 8 and 9 indicate positions on a terminal 6-member aromatic ring;

A, D, X and Y are each a nitrogen;

B is a carbon;

The dotted line in the five-member ring signifies that either the A--B bond or the B--D bond is a double bond;

when A or D is nitrogen and said nitrogen is participating in a double bond, R_1 or R_3, respectively, is a pair of electrons;

when A or D is nitrogen and said nitrogen is not participating in a double bond, R_1 or R_3 is selected from the group consisting of alkyl, alkenyl, alkynyl, cycloalkyl, aryl, hydroxy, alkoxy, halo, C-carboxy, O-carboxy, carbonyl, thiocarbonyl, C-amido, guanyl, sulfonyl and trihalomethane-sulfonyl;

R_5 and R_7 are each independently selected from the group consisting of hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, aryl, hydroxy, alkoxy, halo, C-carboxy, O-carboxy, carbonyl, thiocarbonyl, C-amido, guanyl, sulfonyl, trihalomethane-sulfonyl and a pair of electrons;

R_2 is selected from the group consisting of hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, aryl, hydroxy, alkoxy, halo, C-carboxy, O-carboxy, carbonyl, thiocarbonyl, C-amido, guanyl, sulfonyl and trihalomethane-sulfonyl; or alternatively, R_1 and R_2 or R_2 and R_3 form a 5-7 member ring structure;

R_6 is selected from the group consisting of alkyl, trihaloalkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, sulfinyl, sulfonyl, N-sulfonamido, S-sulfonamido, trihalomethylsulfonamido, carbonyl, thiocarbonyl, C-carboxy, O-carboxy, C-amido, N-amido, cyano, nitro, halo, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, ureido, guanyl, guanidino, amino and a physiologically acceptable salt thereof;

R_4 and R_8 are each independently selected from the group consisting of hydrogen, alkyl, trihaloalkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, sulfinyl, sulfonyl, N-sulfonamido, S-sulfonamido, trihalomethylsulfonamido, carbonyl, thiocarbonyl, C-car-

boxy, O-carboxy, C-amido, N-amido, cyano, nitro, halo, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, ureido, guanyl, guanidino, amino and $-NR_{10}R_{11}$ and, a physiologically acceptable salt;

R_{10} and R_{11} are each independently selected from the group consisting of hydrogen, alkyl, cycloalkyl, aryl, carbonyl and sulfonyl, or alternatively R_{10} and R_{11} form a five- or six-member heteroalicyclic ring; and, a physiologically acceptable salt;

whereas, said R_6 is at position 6;

with the proviso that, for Compound I,

the tyrphostin isomer being capable of inhibiting PDGF receptor kinase activity.

2. The purified tyrphostin isomer of claim 1, wherein

the A--B bond is a double bond,

R_1 and R_2 are each independently selected from the group consisting of alkyl, alkoxy, halogen, aryl, heteroaryl, nitro and amine group or, alternatively R_1 and R_2 form a 5-7 member ring structure;

R_3, R_5 and R_7 are each a pair of electrons;

R_6 is an aryl, selected from the group consisting of phenyl, ferrocene, thiophene, furane, pyrrole, indole, thiazole, imidazole and pyridine.

3. The purified tyrphostin isomer of claim 2, wherein

R_1 and R_2 are each a methyl;

R_4 and R_8 are each a hydrogen.

4. A pharmaceutical composition comprising, as an active ingredient, the purified tyrphostin isomer of claim 1 and a pharmaceutically acceptable carrier.

5. The pharmaceutical composition of claim 4, wherein said pharmaceutically acceptable carrier is a slow release carrier.

6. The pharmaceutical composition of claim 5, wherein said slow release carrier is polylactic acid.

7. Pharmaceutical composition of claim 4 for use as a medicament for treating or preventing a protein tyrosine kinase related disorder in an organism, comprising the step of administering to said organism a therapeutically effective amount of said pharmaceutical composition.

8. Use of a pharmaceutical composition of claim 4 for the manufacture of a medicament for treating or preventing a protein tyrosine kinase related disorder in an organism, comprising the step of administering to said organism a therapeutically effective amount of said pharmaceutical composition.

9. The pharmaceutical composition of claims 7 or 8, wherein said protein tyrosine kinase related disorder is selected from the group consisting of an EGF related disorder, a PDGF related disorder, an BGF related disorder, a PDGF related disorder, an IGF related disorder and a met related disorder.

10. The pharmaceutical composition of claims 7 or 8, wherein said protein tyrosine kinase related disorder is selected from the group consisting of a cell proliferative disorder, a fibrotic disorder and a metabolic disorder.

11. The pharmaceutical composition of claim 10, wherein said cell proliferative disorder is selected from the group consisting of papilloma, blastoglioma, Kaposi's sarcoma, melanoma, lung cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, astrocytoma, head cancer, neck cancer, bladder cancer, breast cancer, lung cancer, colorectal cancer, thyroid cancer, pancreatic cancer, gastric cancer, hepatocellular carcinoma, leukaemia, lymphoma, Hodgkin's disease, Burkitt's disease, arthritis, rheumatoid arthritis, diabetic retinopathy, angiogenesis, restenosis, in-stent restenosis, vascular graft restenosis.

12. The pharmaceutical composition of claim 10, wherein said cell fibrotic disorder is selected from the group consisting of pulmonary fibrosis, hepatic cirrhosis, atherosclerosis, glomerulonephritis, diabetic nephropathy, thrombic microangiopathy syndromes, transplant rejection.

13. The pharmaceutical composition of claim 10, wherein said cell metabolic disorder is selected from the group consisting of psoriasis, diabetes, wound healing, inflammation, and neurodegenerative diseases.

14. The pharmaceutical composition of claims 7 or 8, wherein said protein tyrosine kinase related disorder is selected from the group consisting of papilloma, blastoglioma, Kaposi's sarcoma, melanoma, lung cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, astrocytoma, head cancer, neck cancer, bladder cancer, breast cancer, small-cell lung cancer, colorectal cancer, thyroid cancer, pancreatic cancer, gastric cancer, hepatocellular carcinoma, leukaemia, lymphoma, Hodgkin's disease, Burkitt's disease, psoriasis, pulmonary fibrosis, arthritis, rheumatoid arthritis, diabetic retinopathy, restenosis, in-stent restenosis, vascular graft restenosis, hepatic cirrhosis, atherosclerosis, angiogenesis, glomerulonephritis, diabetic nephropathy, thrombic microangiopathy syndromes, transplant rejection, autoimmune disease, wound healing, inflammation, neurodegenerative diseases, diabetes and hyperimmune disorders.

15. The pharmaceutical composition of claims 7 or 8, wherein said organism is a mammal.

16. The pharmaceutical composition of claim 15, wherein said mammal is a human.

17. Pharmaceutical composition of claim 4 for use as a medicament for locally treating or preventing a disorder of a tissue of an organism comprising the step of locally applying said pharmaceutical composition onto said tissue.

18. Use of a pharmaceutical composition of claim 4 for the manufacture of a medicament for locally treating or preventing a disorder of a tissue of an organism comprising the step of locally applying said pharmaceutical composition onto said tissue.

19. The pharmaceutical composition of claims 17 or 18, wherein said organism is a human.

20. The pharmaceutical composition of claims 17 or 18, wherein said tissue is selected from the group consisting of blood vessel, lung and skin.

21. Purified tyrphostin isomer of claim 1 for use as a medicament for inhibiting cell proliferation comprising the step of subjecting cells to said purified tyrphostin isomer.

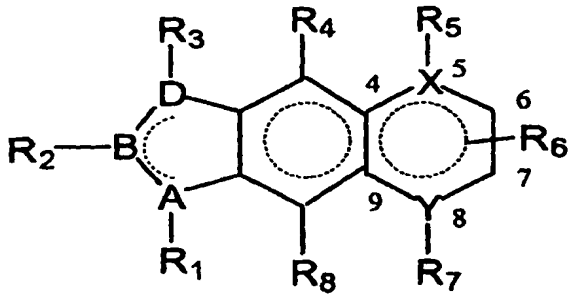
22. Use of pharmaceutical composition of claim 4 for the manufacture of a medicament for inhibiting cell proliferation comprising the step of subjecting cells to said purified tyrphostin isomer.

23. The purified tyrphostin isomer of claims 21 or 22, wherein said cells are of an organism, whereas subjecting the cells to said purified tyrphostin isomer is effected *in vivo*.

24. The purified tyrphostin isomer of claim 23, wherein said organism is a human.

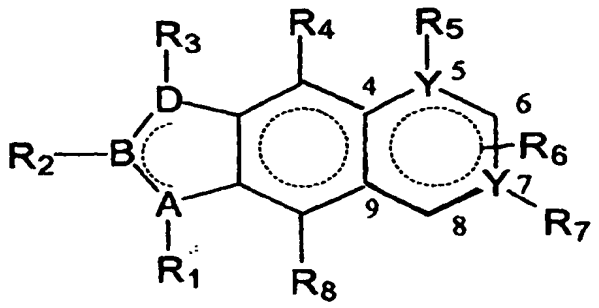
25. Purified tyrphostin isomer of claims 21 or 22, wherein subjecting the said cells to said purified tyrphostin isomer is effected *in vitro*.

26. A method of enriching, for a specific isomer, a tyrphostin of a general formula:



(Compound I)

or



(Compound II)

wherein

4, 5, 6, 7, 8 and 9 indicate positions on a terminal 6-member aromatic ring;

A, D, X and Y are each a nitrogen;

B is a carbon;

The dotted line in the five-member ring signifies that either the A--B bond or the B--D bond is a double bond;

when A or D is nitrogen and said nitrogen is participating in a double bond, R_1 or R_3, respectively, is a pair of electrons;

when A or D is nitrogen and said nitrogen is not participating in a double bond, R_1 or R_3 is selected from the group consisting of alkyl, alkenyl, alkynyl, cycloalkyl, aryl, hydroxy, alkoxy, halo, C-carboxy, O-carboxy, carbonyl, thiocarbonyl, C-amido, guanyl, sulfonyl and trihalomethane-sulfonyl;

R_5 and R_7 are each independently selected from the group consisting of hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, aryl, hydroxy, alkoxy, halo, c-carboxy, O-carboxy, carbonyl, thiocarbonyl, C-amido, guanyl, sulfonyl, trihalomethane-sulfonyl and a pair of electrons;

R_2 is selected from the group consisting of hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, aryl, hydroxy, alkoxy, halo, C-carboxy, O-carboxy, carbonyl, thiocarbonyl, C-amido, guanyl, sulfonyl and trihalomethane-sulfonyl; or alternatively, R_1 and R_2 or R_2 and R_3 form a 5-7 member ring structure;

R_6 is selected from the group consisting of alkyl, trihaloalkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, hydroxy, alkoxy, aryloxy, thiohydroxy thioalkoxy, thioaryloxy, sulfinyl, sulfonyl, N-sulfonamido, S-sulfonamido, trihalomethylsulfonamido, carbonyl, thiocarbonyl, C-carboxy, O-carboxy, C-amido, N-amido, cyano, nitro, halo, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, ureido, guanyl, guanidino, amino and a physiologically acceptable salt or a prodrug thereof;

R_4 et R_8 are each independently selected from the group consisting of hydrogen, alkyl, trihaloalkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, sulfinyl, sulfonyl, N-sulfonamido, S-sulfonamido, trihalomethylsulfonamido, carbonyl, thiocarbonyl, C-carboxy, O-carboxy, C-amido, N-amido,cyano, nitro, halo, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, ureido, guanyl, guanidino, amino and $-NR_{10}R_{11}$ and, a physiologically acceptable salt or a prodrug thereof;

R_{10} and R_{11} are each independently selected from the group consisting of hydrogen, alkyl, cycloalkyl, aryl, carbonyl and sulfonyl, or alternatively R_{10} and R_{11} form a five- or six-member heteroalicyclic ring; and, a physiologically acceptable salt or a prodrug thereof;

whereas, for each molecule of Compound I, R_6 is at position 6 or 7, or, for each molecule of Compound II, R_6 is at position 6 or 8;

the tyrphostin isomer being capable of inhibiting PDGF receptor kinase activity;

the method comprising the steps of:

(i) chromatographing said tyrphostin through a matrix, thereby separating positional isomers of said tyrphostin;

(ii) collecting at least one specific positional isomer of said tyrphostin.

27. The method of claim 26, further comprising the steps of:

(iii) crystallizing said at least one specific positional isomer.

28. The method of claim 26, wherein

the A--B bond is a double bond,

R_1 and R_2 are independently selected from the group consisting of alkyl, alkoxy, halogen, aryl, heteroaryl, nitro and amine group or, alternatively R_1 and R_2 form a 5-7 member ring structure;

R_3, R_5 and R_7 are each a pair of electrons;

R_6 is an aryl, selected from the group consisting of phenyl, ferrocene, thiophene, furane, pyrrole, indole, thiazole, imidazole and pyridine.

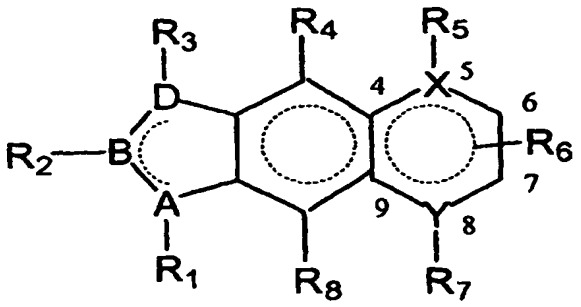
29. The method of claim 28, wherein

R_1 and R_2 are each a methyl;

R_4 and R_8 are each a hydrogen.

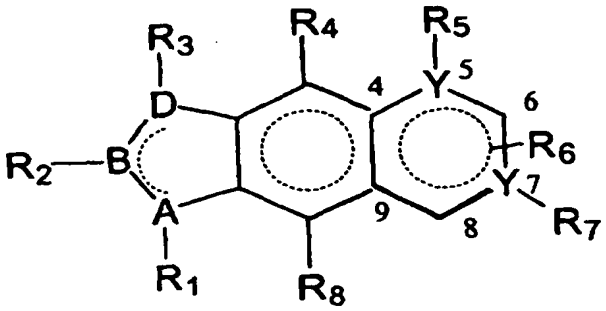
30. A method for preparing a pharmaceutical composition for slow release of a tyrphostin comprising the steps of:

(i) providing purified tyrphostin isomer of a general formula:



(Compound I)

or



(Compound II)

wherein,

4, 5, 6, 7, 8 and 9 indicate positions on a terminal 6-member aromatic ring;

A, D, X and Y are each a nitrogen;

The dotted line in the five-member ring signifies that either the A--B bond or the B--D bond is a double bond;

when A or D is nitrogen and said nitrogen is participating in a double bond, R_1 or R_3, respectively, is a pair of electrons;

when A or D is nitrogen and said nitrogen is not participating in a double bond, R_1 or R_3 is selected from the group consisting of alkyl, alkenyl, alkynyl, cycloalkyl, aryl, hydroxy, alkoxy, halo, C-carboxy, O-carboxy,

carbonyl, thiocarbonyl, C-amido, guanyl, sulfonyl and trihalomethane-sulfonyl;

R_5 and R_7 are each independently selected from the group consisting of hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, aryl, hydroxy, alkoxy, halo, C-carboxy, O-carboxy, carbonyl, thiocarbonyl, C-amido, guanyl, sulfonyl, trihalomethane-sulfonyl and a pair of electrons;

R_2 is selected from the group consisting of hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, aryl, hydroxy, alkoxy, halo, C-carboxy, O-carboxy, carbonyl, thiocarbonyl, C-amido, guanyl, sulfonyl and trihalomethane-sulfonyl; or

alternatively, R_1 and R_2 or R_2 form a 5-7 member ring structure;

R_6 is selected from the group consisting of alkyl, trihaloalkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, sulfinyl, sulfonyl, N-sulfonamido, S-sulfonamido, trihalomethylsulfonamido, carbonyl, thiocarbonyl, C-carboxy, O-carboxy, C-amido, N-amido, cyano, nitro, halo, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, ureido, guanyl, guanidino amino and a physiologically acceptable salt or a prodrug thereof;

R_4 and R_8 are each independently selected from the group consisting of hydrogen, alkyl, trihaloalkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, sulfinyl, sulfonyl, N-sulfonamido, S-sulfonamido, trihalomethylsulfonamido, carbonyl, thiocarbonyl, C-carboxy, O-carboxy, C-amido, N-amido, cyano, nitro, halo, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, ureido, guanyl, guanidino, amino and $-NR_{10}R_{11}$ and, a physiologically acceptable salt or a prodrug thereof;

R_{10} and R_{11} are each independently selected from the group consisting of hydrogen, alkyl, cycloalkyl, aryl, carbonyl and sulfonyl, or alternatively R_{10} and R_{11} form a five- or six-member heteroalicyclic ring; and, a physiologically acceptable salt or a prodrug thereof,

whereas, said R_6 is at position 6;

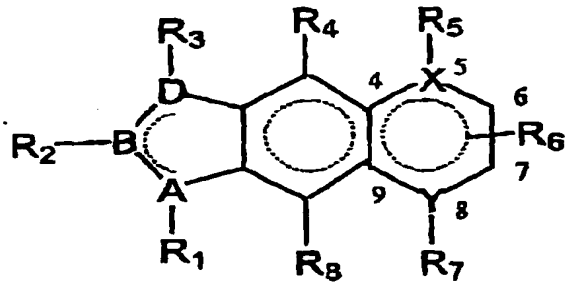
(ii) dissolving or dispersing a slow release carrier and said purified tyrphostin isomer in an organic solvent for obtaining an organic solution containing said carrier and said purified tyrphostin isomer;

(iii) adding said organic solution into an aqueous solution for obtaining an oil-in-water-type emulsion; and

(iv) evaporating said organic solvent from said oil-in-water-type emulsion for obtaining a colloidal suspension of particles containing said slow release carrier and said purified tyrphostin isomer.

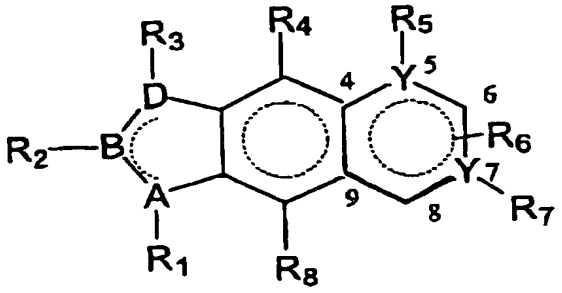
31. The method of claim 30, wherein said slow release carrier is polylactic acid.

32. A stent comprising a substantially tubular body, the body is made of a material designed for slow release of a purified tyrphostin isomer of a general formula:



(Compound I)

or



(Compound II)

wherein,

4, 5, 6, 7, 8 and 9 indicate positions on a terminal 6-member ring;

A, D, X and Y are each a nitrogen;

The dotted line in the five-member ring signifies that either the A--B bond or the B--D bond is a double bond;

when A or D is nitrogen and said nitrogen is participating in a double bond, R_1 or R_3, respectively, is a pair of electrons;

when A or D is nitrogen and said nitrogen is not participating in a double bond, R_1 or R_3 is selected from the group consisting of alkyl, alkenyl, alkynyl, cycloalkyl, aryl, hydroxy, alkoxy, halo, C-carboxy, O-carboxy, carbonyl, thiocarbonyl, C-amido, guanyl, sulfonyl and trihalomethane-sulfonyl;

R_5 and R_7 are each independently selected from the group consisting of hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, aryl, hydroxy, alkoxy, halo, C-carboxy, O-carboxy, carbonyl, thiocarbonyl, C-amido, guanyl, sulfonyl, trihalomethane-sulfonyl and a pair of electrons;

R_2 is selected from the group consisting of hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, aryl, hydroxy, alkoxy, halo, c-carboxy, O-carboxy, carbonyl, thiocarbonyl, C-amido, guanyl, sulfonyl and trihalamethane-sulfonyl; or alternatively, R_1 and R_2 or R_2 and R_3 form a 5-7 member ring structure;

R_6 is selected from the group consisting of alkyl, trihaloalkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, sulfinyl, sulfonyl, N-sulfonamido, S-sulfonamido, trihalomethylsulfonamido, carbonyl, thiocarbonyl, C-carboxy, O-carboxy, C-amido, N-amido, cyano, nitro, halo, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, ureido, guanyl, guanidino, amino and a physiologically acceptable salt or a prodrug thereof;

R_4 and R_8 are each independently selected from the group consisting of hydrogen, alkyl, trihaloalkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, sulfinyl, sulfonyl, N-sulfonamido, S-sulfonamido, trihalomethylsulfonamido, carbonyl, thiocarbonyl, C-carboxy, O-carboxy, C-amido, N-amido, cyano, nitro, halo, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, ureido, guanyl, guanidino, amino and $-NR_{10}R_{11}$ and, a physiologically acceptable salt or a prodrug thereof;

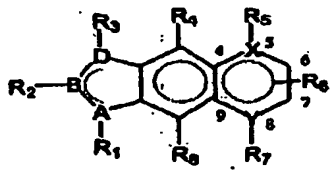
R_{10} and R_{11} are each independently selected from the group consisting of hydrogen, alkyl, cycloalkyl, aryl, carbonyl and sulfonyl, or alternatively R_{10} and R_{11} form a five- or six-member heteroalicyclic ring; and, a physiologically acceptable salt or a prodrug thereof;

whereas, said R_6 is at position 6;

the tyrphostin isomer being capable of inhibiting PDGF receptor kinase activity.

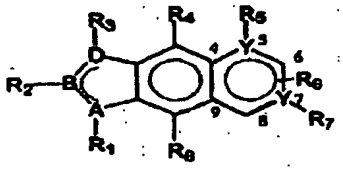
Patentansprüche

1. Ein im Wesentlichen gereinigtes Tyrphostin-Isomer mit einer allgemeinen Formel:



(Verbindung I)

oder



(Verbindung II)

wobei
4, 5, 6, 7, 8 und 9 Positionen an einem endständigen 6-gliedrigen Aromatenring andeuten;
A, D, X und Y jedes ein Stickstoff sind;
B ein Kohlenstoff ist;
die gepunktete Linie in dem fünfgliedrigen Ring anzeigt, dass entweder die A--B-Bindung oder die B--D-Bindung eine Doppelbindung ist;
wenn A oder D Stickstoff ist und besagter Stickstoff an einer Doppelbindung teilnimmt, R_1 beziehungsweise R_3 ein Elektronenpaar ist;
wenn A oder D Stickstoff ist und besagter Stickstoff nicht an einer Doppelbindung teilnimmt, R_1 oder R_3 aus der aus Alkyl, Alkenyl, Alkynyl, Cycloalkyl, Aryl, Hydroxy, Alkoxy, Halo, C-Carboxy, O-Carboxy, Carbonyl, Thiocarbonyl, C-Amido, Guanyl, Sulfonyl und Trihalomethansulfonyl bestehenden Gruppe gewählt ist;
R_5 und R_7 jeweils unabhängig aus der aus Wasserstoff, Alkyl, Alkenyl, Alkynyl, Cycloalkyl, Aryl, Hydroxy, Alkoxy, Halo, C-Carboxy, O-Carboxy, Carbonyl, Thiocarbonyl, C-Amido, Guanyl, Sulfonyl, Trihalomethansulfonyl und einem Elektronenpaar bestehenden Gruppe gewählt sind;
R_2 aus der Gruppe gewählt ist, bestehend aus Wasserstoff, Alkyl, Alkenyl, Alkynyl, Cycloalkyl, Aryl, Hydroxy, Alkoxy, Halo, C-Carboxy, O-Carboxy, Carbonyl, Thiocarbonyl, C-Amido, Guanyl, Sulfonyl und Trihalomethansulfonyl;
oder alternativ R_1 und R_2 oder R_2 und R_3 eine 5-7-gliedrige Ringstruktur bilden;
R_6 aus der Gruppe gewählt ist, bestehend aus Alkyl, Trihaloalkyl, Cycloalkyl, Alkenyl, Alkynyl, Aryl, Heteroaryl, Heteroalicyclisch, Hydroxy, Alkoxy, Aryloxy, Thiohydroxy, Thioalkoxy, Thioaryloxy, Sulfinyl, Sulfonyl, N-Sulfonamido, S-Sulfonamido, Trihalomethylsulfonamido, Carbonyl, Thiocarbonyl, C-Carboxy, O-Carboxy, C-Amido, N-Amido, Cyano, Nitro, Halo, O-Carbamyl, N-Carbamyl, O-Thiocarbamyl, N-Thiocarbamyl, Ureido, Guanyl, Guanidino, Amino und einem physiologisch akzeptablen Salz davon;
R_4 und R_8 jedes unabhängig aus der Gruppe gewählt sind, bestehend aus Wasserstoff, Alkyl, Trihaloalkyl, Cycloalkyl, Alkenyl, Alkynyl, Aryl, Heteroaryl, Heteroalicyclisch, Hydroxy, Alkoxy, Aryloxy, Thiohydroxy, Thioalkoxy, Thioaryloxy, Sulfinyl, Sulfonyl, N-Sulfonamido, S-Sulfonamido, Trihalomethylsulfonamido, Carbonyl, Thiocarbonyl, C-Carboxy, 0-Carboxy, C-Amido, N-Amido, Cyano, Nitro, Halo, O-Carbamyl, N-Carbamyl, O-Thiocarbamyl, N-thiocarbamyl, Ureido, Guanyl, Guanidino, Amino und $-NR_{10}R_{11}$ und einem physiologisch akzeptablen Salz;
R_{10} und R_{11} jedes unabhängig aus der Gruppe gewählt sind, bestehend aus Wasserstoff, Alkyl, Cycloalkyl, Aryl, Carbonyl und Sulfonyl, oder alternativ bilden R_{10} und R_{11} einen fünf- oder sechsgliedrigen heteroalicyclischen Ring; und einem physiologisch akzeptablen Salz;
während besagtes R_6 an Position 6 steht;
unter dem Vorbehalt, dass, für Verbindung I,
das Tyrphostin-Isomer in der Lage ist, PDGF-Rezeptorkinaseaktivität zu inhibieren.

2. Das gereinigte Tyrphostin-Isomer gemäß Anspruch 1,
wobei
die A--B-Bindung eine Doppelbindung ist;

R_1 und R_2 jedes unabhängig aus der Gruppe gewählt sind, bestehend aus Alkyl, Alkoxy, Halogen, Aryl, Heteroaryl, Nitro und Amingruppe, oder alternativ R_1 und R_2 eine 5-7-gliedrige Ringstruktur bilden;
R_3, R_5 und R_7 jedes ein Elektronenpaar sind;
R_6 ein Aryl ist, gewählt aus der aus Phenyl, Ferrocen, Thiophen, Furan, Pyrrol, Indol, Thiazol, Imidazol und Pyridin bestehenden Gruppe.

3. Das gereinigte Tyrphostin-Isomer gemäß Anspruch 2,
wobei
R_1 und R_2 jedes ein Methyl sind;
R_4 und R_8 jedes ein Wasserstoff sind.

4. Eine pharmazeutische Zusammensetzung, welche als aktiven Bestandteil das gereinigte Tyrphostin-Isomer gemäß Anspruch 1 und einen pharmazeutisch akzeptablen Träger umfasst.

5. Die pharmazeutische Zusammensetzung gemäß Anspruch 4, wobei besagter pharmazeutisch akzeptable Träger ein Träger mit langsamer Freisetzung ist.

6. Die pharmazeutische Zusammensetzung gemäß Anspruch 5, wobei besagter Träger mit langsamer Freisetzung Polymilchsäure ist.

7. Pharmazeutische Zusammensetzung gemäß Anspruch 4 zur Anwendung als Medikament zur Behandlung oder Verhütung einer mit Proteintyrosinkinase zusammenhängenden Störung in einem Organismus, welche den Schritt des Verabreichens einer therapeutisch effektiven Menge besagter pharmazeutischer Zusammensetzung an besagten Organismus umfasst.

8. Anwendung einer pharmazeutischen Zusammensetzung gemäß Anspruch 4 zur Herstellung eines Medikaments zur Behandlung oder Verhütung einer mit Proteintyrosinkinase zusammenhängenden Störung in einem Organismus, welche den Schritt des Verabreichens einer therapeutisch effentiven Menge besagter pharmazeutischer Zusammensetzung an besagten Organismus umfasst.

9. Die pharmazeutische Zusammensetzung gemäß Ansprüchen 7 oder 8, wobei besagte mit Proteintyrosinkinase zusammenhängende Störung aus der aus einer mit EGF zusammenhängenden Störung, einer mit PDGF zusammenhängenden Störung, einer mit EGF zusammenhängenden Störung, einer mit PDGF zusammenhängenden Störung, einer mit IGF zusammenhängenden Störung und einer mit Met zusammenhängenden Störung bestehenden Gruppe gewählt ist.

10. Die pharmazeutische Zusammensetzung gemäß Ansprüchen 7 oder 8, wobei besagte mit Proteintyrosinkinase zusammenhängende Störung aus der aus einer Zellwucherungsstörung, einer fibrotischen Störung und einer Metabolismusstörung bestehenden Gruppe gewählt ist.

11. Die pharmazeutische Zusammensetzung gemäß Anspruch 10, wobei besagte Zellwucherungsstörung aus der aus Papillom, Blastogliom, Kaposi-Sarkom, Melanom, Lungenkrebs, Eierstockkrebs, Prostatakrebs, schuppenförmigem Zellkarzinom, Astrocytom, Kopfkrebs, Halskrebs, Blasenkrebs, Brustkrebs, Lungenkrebs, Dickdarm-/Mastdarmkrebs, Schilddrüsenkrebs, Bauchspeicheldrüsenkrebs, Magenkrebs, hepatozellulärem Karzinom, Leukämie, Lymphom, Morbus Hodgkin, Morbus Burkitt, Arthritis, rheumatoider Arthritis, diabetischer Retinopathie, Angiogenese, Restenose, In-Stent-Restenose, Gefäßtransplantat-Restenose bestehenden Gruppe gewählt ist.

12. Die pharmazeutische Zusammensetzung gemäß Anspruch 10, wobei besagte fibrotische Zellstörung aus der aus pulmonärer Fibrose, hepatischer Zirrhose, Atherosklerose, Glomerulonephritis, diabetischer Nephropathie, thrombischen Mikroangiopathiesyndromen, Transplantatabstoßung bestehenden Gruppe gewählt ist.

13. Die pharmazeutische Zusammensetzung gemäß Anspruch 10, wobei besagte Zellmetabolismusstörung aus der aus Psoriasis, Diabetes, Wundheilung, Entzündung und neurodegenerativen Krankheiten bestehenden Gruppe gewählt ist.

14. Die pharmazeutische Zusammensetzung gemäß Ansprüchen 7 oder 8, wobei besagte mit Proteintyrosinkinase zusammenhängende Störung aus der aus Papillom, Blastogliom, Kaposi-Sarkom, Melanom, Lungenkrebs, Eierstockkrebs, Prostatakrebs, schuppenförmigem Zellkarzinom, Astrocytom, Kopfkrebs, Halskrebs, Blasenkrebs,

Brustkrebs, Kleinzellen-Lungenkrebs, Dickdarm-/Mastdarmkrebs, Schilddrüsenkrebs, Bauchspeicheldrüsenkrebs, Magenkrebs, hepatozellulärem Karzinom, Leukämie, Lymphom, Morbus Hodgkin, Morbus Burkitt, Psoriase, pulmonärer Fibrose, Arthritis, rheumatoider Arthritis, diabetischer Retinopathie, Restenose, In-Stent-Restenose, Gefäßtransplantat-Restenose, hepatischer Zirrhose, Atherosklerose, Angiogenese, Glomerulonephritis, diabetischer Nekropathie, thrombischen Mikroangiopathiesyndromen, Transplantatabstoßung, Autoimmunkrankheit, Wundheilung, Entzündung, neurodegenerativen Krankheiten, Diabetes und Hyperimmunkrankheiten bestehenden Gruppe gewählt ist.

15. Die pharmazeutische Zusammensetzung gemäß Ansprüchen 7 oder 8, wobei besagter Organismus ein Säugetier ist.

16. Die pharmazeutische Zusammensetzung gemäß Anspruch 15, wobei besagtes Säugetier ein Mensch ist.

17. Pharmazeutische Zusammensetzung gemäß Anspruch 4 zur Anwendung als ein Medikament zur lokalen Behandlung oder Verhütung einer Störung eines Gewebes eines Organismus, welche den Schritt des lokalen Anwendens besagter pharmazeutischer Zusammensetzung auf besagtes Gewebe umfasst.

18. Anwendung einer pharmazeutischen Zusammensetzung gemäß Anspruch 4 zur Herstellung eines Medikaments zur lokalen Behandlung oder Verhütung einer Störung eines Gewebes eines Organismus, welche den Schritt des lokalen Anwendens besagter pharmazeutischer Zusammensetzung auf besagtes Gewebe umfasst.

19. Die pharmazeutische Zusammensetzung gemäß Ansprüchen 17 oder 18, wobei besagter Organismus ein Mensch ist.

20. Die pharmazeutische Zusammensetzung gemäß Ansprüchen 17 oder 18, wobei besagtes Gewebe aus der aus Blutgefäß, Lunge und Haut bestehenden Gruppe gewählt ist.

21. Gereinigtes Tyrphostin-Isomer gemäß Anspruch 1 zur Anwendung als ein Medikament zur Inhibierung von Zellwucherung, welche Anwendung den Schritt des besagtem gereinigten Tyrphostin-Isomer Aussetzens von Zellen umfasst.

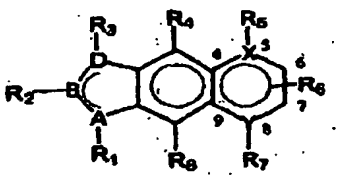
22. Anwendung der pharmazeutischen Zusammensetzung gemäß Anspruch 4 zur Herstellung eines Medikaments zur Inhibierung von Zellwucherung, welche Anwendung den Schritt des besagtem gereinigten Tyrphostin-Isomer Aussetzens von Zellen umfasst.

23. Das gereinigte Tyrphostin-Insomer gemäß Ansprüchen 21 oder 22, wobei besagte Zellen von einem Organismus stammen, während das besagtem gereinigten Tyrphostin-Isomer Aussetzen der Zellen *in vivo* bewerkstelligt wird.

24. Das gereinigte Tyrphostin-Isomer gemäß Anspruch 23, wobei besagter Organismus ein Mensch ist.

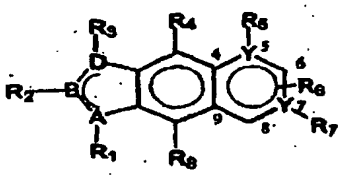
25. Gereinigtes Tyrphostin-Isomer gemäß Ansprüchen 21 oder 22, wobei das besagtem gereinigten Tyrphostin-Isomer Aussetzen der besagten Zellen *in vitro* bewerkstelligt wird.

26. Ein Verfahren zum Anreichern, für ein spezifisches Isomer, eines Tyrphostins einer allgemeinen Formel:



(Verbindung I)

oder



(Verbindung II)

wobei
4, 5, 6, 7, 8 und 9 Positionen an einem endständigen 6-gliedrigen Aromatenring andeuten;
A, D, X und Y jedes ein Stickstoff sind;
B ein Kohlenstoff ist;
die gepunktete Linie in dem fünfgliedrigen Ring anzeigt, dass entweder die A--B-Bindung oder die B--D-Bindung eine Doppelbindung ist;
wenn A oder D Stickstoff ist und besagter Stickstoff an einer Doppelbindung teilnimmt, R_1 beziehungsweise R_3 ein Elektronenpaar ist;
wenn A oder D Stickstoff ist und besagter Stickstoff nicht an einer Doppelbindung teilnimmt, R_1 oder R_3 aus der aus Alkyl, Alkenyl, Alkynyl, Cycloalkyl, Aryl, Hydroxy, Alkoxy, Halo, C-Carboxy, O-Carboxy, Carbonyl, Thiocarbonyl, C-Amido, Guanyl, Sulfonyl und Trihalomethansulfonyl bestehenden Gruppe gewählt ist;
R_5 und R_7 jeweils unabhängig aus der Gruppe gewählt sind, bestehend aus Wasserstoff, Alkyl, Alkenyl, Alkynyl, Cycloalkyl, Aryl, Hydroxy, Alkoxy, Halo, C-Carboxy, O-Carboxy, Carbonyl, Thiocarbonyl, C-Amido, Guanyl, Sulfonyl, Trihalomethansulfonyl und einem Elektronenpaar;
R_2 aus der Gruppe gewählt ist, bestehend aus Wasserstoff, Alkyl, Alkenyl, Alkynyl, Cycloalkyl, Aryl, Hydroxy, Alkoxy, Halo, C-Carboxy, O-Carboxy, Carbonyl, Thiocarbonyl, C-Amido, Guanyl, Sulfonyl und Trihalomethansulfonyl; oder alternativ R_1 und R_2 oder R_2 und R_3 eine 5-7-gliedrige Ringstruktur bilden;
R_6 aus der Gruppe gewählt ist, bestehend aus Alkyl, Trihaloalkyl, Cycloalkyl, Alkenyl, Alkynyl, Aryl, Heteroaryl, Heteroalicyclisch, Hydroxy, Alkoxy, Aryloxy, Thiohydroxy, Thioalkoxy, Thioaryloxy, Sulfinyl, Sulfonyl, N-Sulfonamido, S-Sulfonamido, Trihalomethylsulfonamido, Carbonyl, Thiocarbonyl, C-Carboxy, O-Carboxy, C-Amido, N-Amido, Cyano, Nitro, Halo, O-Carbamyl, N-Carbamyl, O-Thiocarbamyl, N-Thiocarbamyl, Ureido, Guanyl, Guanidino, Amino und einem physiologisch akzeptablen Salz oder einem Vor-Arzneistoff davon;
R_4 und R_8 jedes unabhängig aus der Gruppe gewählt sind, bestehend aus Wasserstoff, Alkyl, Trihaloalkyl, Cycloalkyl, Alkenyl, Alkynyl, Aryl, Heteroaryl, Heteroalicyclisch, Hydroxy, Alkoxy, Aryloxy, Thiohydroxy, Thioalkoxy, Thioaryloxy, Sulfinyl, Sulfonyl, N-Sulfonamido, S-Sulfonamido, Trihalomethylsulfonamido, Carbonyl, Thiocarbonyl, C-Carboxy, O-Carboxy, C-Amido, N-Amido, Cyano, Nitro, Halo, O-Carbamyl, N-Carbamyl, O-Thiocarbamyl, N-thiocarbamyl, Ureido, Guanyl, Guanidino, Amino und $-NR_{10}R_{11}$ und einem physiologisch akzeptablen Salz oder einem Vor-Arzneistoff davon;
R_{10} und R_{11} jedes unabhängig aus der Gruppe gewählt sind, bestehend aus Wasserstoff, Alkyl, Cycloalkyl, Aryl, Carbonyl und Sulfonyl, oder alternativ R_{10} und R_{11} einen fünf- oder sechsgliedrigen heteroalicyclischen Ring bilden; und einem physiologisch akzeptablen Salz oder einem Vor-Arzneistoff davon;
während, für jedes Molekül von Verbindung I, R_6 an Position 6 oder 7 steht, oder, für jedes Molekül von Verbindung II, R_6 an Position 6 oder 8 steht;
wobei das Tyrphostin-Isomer in der Lage ist, PDGF-Rezeptorkinaseaktivität zu inhibieren;
wobei das Verfahren die Schritte umfasst des:

(i) Chromatographierens besagten Tyrphostins durch eine Matrix, wodurch positionale Isomere besagten Tyrphostins abgeschieden werden;
(ii) Sammelns zumindest eines spezifischen positionalen Isomers besagten Tyrphostins.

27. Das Verfahren gemäß Anspruch 26, das weiter die Schritte umfasst des:

(iii) Kristallisierens besagten zumindest einen spezifischen positionalen Isomers.

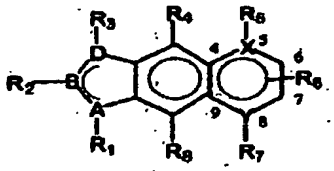
28. Das Verfahren gemäß Anspruch 26, wobei die A--B-Bindung eine Doppelbindung ist,
R_1 und R_2 jedes unabhängig aus der aus Alkyl, Alkoxy, Halogen, Aryl, Heteroaryl, Nitro und Amingruppe bestehenden Gruppe gewählt ist oder alternativ R_1 und R_2 eine 5-7-gliedrige Ringstruktur bilden;
R_3, R_5 und R_7 jedes ein Elektronenpaar sind;

R_6 ein Aryl ist, gewählt aus der aus Phenyl, Ferrocen, Thiophen, Furan, Pyrrol, Indol, Thiazol, Imidazol und Pyridin bestehenden Gruppe.

29. Das Verfahren gemäß Anspruch 28, wobei
R_1 und R_2 jedes ein Methyl sind;
R_4 und R_8 jedes ein Wasserstoff sind.

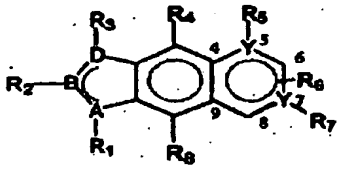
30. Ein Verfahren zur Herstellung einer pharmazeutischen Zusammensetzung zur langsamen Freisetzung eines Tyrphostins, welches die Schritte umfasst des:

(i) Verschaffens gereinigten Tyrphostin-Isomers einer allgemeinen Formel:



(Verbindung I)

oder



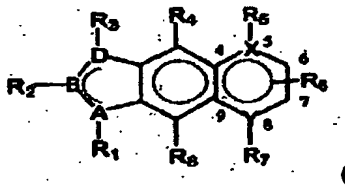
(Verbindung II)

wobei
4, 5, 6, 7, 8 und 9 Positionen an einem endständigen 6-gliedrigen Aromatenring andeuten;
A, D, X und Y jedes ein Stickstoff sind;
die gepunktete Linie in dem fünfgliedrigen Ring anzeigt, dass entweder die A--B-Bindung oder die B--D-Bindung eine Doppelbindung ist;
wenn A oder D Stickstoff ist und besagter Stickstoff an einer Doppelbindung teilnimmt, R_1 beziehungsweise R_3 ein Elektronenpaar ist;
wenn A oder D Stickstoff ist und besagter Stickstoff nicht an einer Doppelbindung teilnimmt, R_1 oder R_3 aus der aus Alkyl, Alkenyl, Alkynyl, Cycloalkyl, Aryl, Hydroxy, Alkoxy, Halo, C-Carboxy, O-Carboxy, Carbonyl, Thiocarbonyl, C-Amido, Guanyl, Sulfonyl und Trihalomethansulfonyl bestehenden Gruppe gewählt ist;
R_5 und R_7 jedes unabhängig aus der Gruppe gewählt sind, bestehend aus Wasserstoff, Alkyl, Alkenyl, Alkynyl, Cycloalkyl, Aryl, Hydroxy, Alkoxy, Halo, C-Carboxy, O-Carboxy, Carbonyl, Thiocarbonyl, C-Amido, Guanyl, Sulfonyl, Trihalomethansulfonyl und einem Elektronenpaar;
R_2 aus der Gruppe gewählt ist, bestehend aus Wasserstoff, Alkyl, Alkenyl, Alkynyl, Cycloalkyl, Aryl, Hydroxy, Alkoxy, Halo, C-Carboxy, O-Carboxy, Carbonyl, Thiocarbonyl, C-Amido, Guanyl, Sulfonyl und Trihalomethansulfonyl; oder
alternativ R_1 und R_2 oder R_2 eine 5-7-gliedrige Ringstruktur bilden;
R_6 aus der Gruppe gewählt ist, bestehend aus Alkyl, Trihaloalkyl, Cycloalkyl, Alkenyl, Alkynyl, Aryl, Heteroaryl, Heteroalicyclisch, Hydroxy, Alkoxy, Aryloxy, Thiohydroxy, Thioalkoxy, Thioaryloxy, Sulfinyl, Sulfonyl, N-Sulfonamido, S-Sulfonamido, Trihalomethylsulfonamido, Carbonyl, Thiocarbonyl, C-Carboxy, O-Carboxy, C-Amido, N-Amido, Cyano, Nitro, Halo, O-Carbamyl, N-Carbamyl, O-Thiocarbamyl, N-Thiocarbamyl, Ureido, Guanyl, Guanidino, Amino und einem physiologisch akzeptablen Salz oder einem Vor-Arzneistoff davon;
R_4 und R_8 jedes unabhängig aus der Gruppe gewählt sind, bestehend aus Wasserstoff, Alkyl, Trihaloalkyl, Cycloalkyl, Alkenyl, Alkynyl, Aryl, Heteroaryl, Heteroalicyclisch, Hydroxy, Alkoxy, Aryloxy, Thiohydroxy, Thioalkoxy, Thioaryloxy, Sulfinyl, Sulfonyl, N-Sulfonamido, S-Sulfonamido, Trihalomethylsulfonamido, Carbonyl,

Thiocarbonyl, C-Carboxy, O-Carboxy, C-Amido, N-Amido, Cyano, Nitro, Halo, O-Carbamyl, N-Carbamyl, O-Thiocarbamyl, N-thiocarbamyl, Ureido, Guanyl, Guanidino, Amino und $-NR_{10}R_{11}$ und einem physiologisch akzeptablen Salz oder einem Vor-Arzneistoff davon;
R_{10} und R_{11} jedes unabhängig aus der Gruppe gewählt sind, bestehend aus Wasserstoff, Alkyl, Cycloalkyl, Aryl, Carbonyl und Sulfonyl, oder alternativ R_{10} und R_{11} einen fünf- oder sechsgliedrigen heteroalicyclischen Ring bilden; und einem physiologisch akzeptablen Salz oder einem Vor-Arzneistoff davon;
während besagtes R_6 an Position 6 steht;
(ii) Auflösens oder Dispergierens eines Trägers für langsame Freisetzung und besagten gereinigten Tyrphostin-Isomers in einem organischen Lösungsmittel, um eine organische Lösung zu erhalten, die besagten Träger und besagtes gereinigtes Tyrphostin-Isomer enthält;
(iii) Zusetzens besagter organischer Lösung zu einer wässrigen Lösung, um eine Emulsion vom Öl-in-Wasser-Typ zu erhalten; und
(iv) Verdampfens besagten organischen Lösungsmittels aus besagter Emulsion vom Öl-in-Wasser-Typ, um eine Kolloidsuspension von Partikeln, die besagten Träger für langsame Freisetzung und besagtes gereinigtes Tyrphostin-Isomer enthält, zu erhalten.

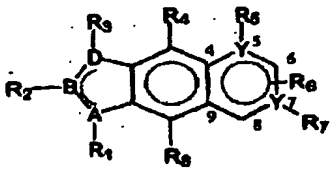
31. Das Verfahren gemäß Anspruch 30, wobei besagter Träger für langsame Freisetzung Polymilchsäure ist.

32. Ein Stent, der einen im Wesentlichen röhrenförmigen Körper umfasst, welcher Körper aus einem Material hergestellt ist, das entworfen ist zur langsamen Freisetzung eines gereinigten Tyrphostin-Isomers einer allgemeinen Formel:



(Verbindung I)

oder



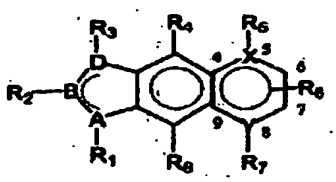
(Verbindung II)

wobei
4, 5, 6, 7, 8 und 9 Positionen an einem endständigen 6-gliedrigen Aromatenring andeuten;
A, D, X und Y jedes ein Stickstoff sind;
die gepunktete Linie in dem fünfgliedrigen Ring anzeigt, dass entweder die A--B-Bindung oder die B--D-Bindung eine Doppelbindung ist;
wenn A oder D Stickstoff ist und besagter Stickstoff an einer Doppelbindung teilnimmt, R_1 beziehungsweise R_3 ein Elektronenpaar ist;
wenn A oder D Stickstoff ist und besagter Stickstoff nicht an einer Doppelbindung teilnimmt, R_1 oder R_3 aus der aus Alkyl, Alkenyl, Alkynyl, Cycloalkyl, Aryl, Hydroxy, Alkoxy, Halo, C-Carboxy, O-Carboxy, Carbonyl, Thiocarbonyl, C-Amido, Guanyl, Sulfonyl und Trihalomethansulfonyl bestehenden Gruppe gewählt ist;
R_5 und R_7 jeweils unabhängig aus der aus Wasserstoff, Alkyl, Alkenyl, Alkynyl, Cycloalkyl, Aryl, Hydroxy, Alkoxy, Halo, C-Carboxy, O-Carboxy, Carbonyl, Thiocarbonyl, C-Amido, Guanyl, Sulfonyl, Trihalomethansulfonyl und einem Elektronenpaar bestehenden Gruppe gewählt sind;
R_2 aus der Gruppe gewählt ist, bestehend aus Wasserstoff, Alkyl, Alkenyl, Alkynyl, Cycloalkyl, Aryl, Hydroxy, Alk-

oxy, Halo, C-Carboxy, O-Carboxy, Carbonyl, Thiocarbonyl, C-Amido, Guanyl, Sulfonyl und Trihalomethansulfonyl; oder
alternativ bilden R_1 und R_2 oder R_2 und R_3 eine 5-7-gliedrige Ringstruktur;
R_6 aus der Gruppe gewählt ist, bestehend aus Alkyl, Trihaloalkyl, Cycloalkyl, Alkenyl, Alkynyl, Aryl, Heteroaryl, Heteroalicyclisch, Hydroxy, Alkoxy, Aryloxy, Thiohydroxy, Thioalkoxy, Thioaryloxy, Sulfinyl, Sulfonyl, N-Sulfonamido, S-Sulfonamido, Trihalomethylsulfonamido, Carbonyl, Thiocarbonyl, C-Carboxy, O-Carboxy, C-Amido, N-Amido, Cyano, Nitro, Halo, O-Carbamyl, N-Carbamyl, O-Thiocarbamyl, N-Thiocarbamyl, Ureido, Guanyl, Guanidino, Amino und einem physiologisch akzeptablen Salz oder einem Vor-Arzneistoff davon;
R_4 und R_8 jedes unabhängig aus der Gruppe gewählt sind, bestehend aus Wasserstoff, Alkyl, Trihaloalkyl, Cycloalkyl, Alkenyl, Alkynyl, Aryl, Heteroaryl, Heteroalicyclisch, Hydroxy, Alkoxy, Aryloxy, Thiohydroxy, Thioalkoxy, Thioaryloxy, Sulfinyl, Sulfonyl, N-Sulfonamido, S-Sulfonamido, Trihalomethylsulfonamido, Carbonyl, Thiocarbonyl, C-Carboxy, O-Carboxy, C-Amido, N-Amido, Cyano, Nitro, Halo, O-Carbamyl, N-Carbamyl, O-Thiocarbamyl, N-thiocarbamyl, Ureido, Guanyl, Guanidino, Amino und $-NR_{10}R_{11}$ und einem physiologisch akzeptablen Salz oder einem Vor-Arzneistoff davon;
R_{10} und R_{11} jedes unabhängig aus der Gruppe gewählt sind, bestehend aus Wasserstoff, Alkyl, Cycloalkyl, Aryl, Carbonyl und Sulfonyl, oder alternativ bilden R_{10} und R_{11} einen fünf- oder sechsgliedrigen heteroalicyclischen Ring; und einem physiologisch akzeptablen Salz oder einem Vor-Arzneistoff davon;
während besagtes R_6 an Position 6 steht;
wobei das Tyrphostin-Isomer in der Lage ist, PDGF-Rezeptorkinaseaktivität zu inhibieren.

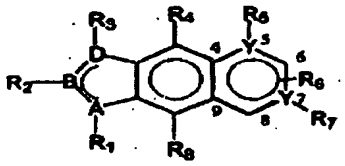
Revendications

1. Isomère de tyrphostine essentiellement purifié répondant à la formule générale :



(composé I)

ou



(composé II)

dans laquelle
les chiffres 4, 5, 6, 7, 8 et 9 indiquent des positions sur un noyau aromatique hexagonal terminal ;
A, D, X et Y représentent chacun un atome d'azote ;
B représente un atome de carbone ;
la ligne en pointillé dans le noyau pentagonal signifie que, soit la liaison A--B, soit la liaison B--D est une liaison double ;
lorsque A ou D représente un atome d'azote et lorsque ledit atome d'azote fait partie d'une liaison double, R_1 ou R_3, respectivement représente une paire d'électrons ;
lorsque A ou D représente un atome d'azote et lorsque ledit atome d'azote ne fait pas partie d'une liaison double, R_1 ou R_3 est choisi parmi le groupe constitué par un groupe alkyle, un groupe alcényle, un groupe alcynyle, un groupe cycloalkyle, un groupe aryle, un groupe hydroxyle, un groupe alcoxy, un atome d'halogène, un groupe C-carboxyle, un groupe O-carboxyle, un groupe carbonyle, un groupe thiocarbonyle, un groupe C-amido, un grou-

pe guanyle, un groupe sulfonyle et un groupe trihalogénométhane-sulfonyle ;

R_5 et R_7 sont choisis, chacun indépendamment l'un de l'autre, parmi le groupe constitué par un atome d'hydrogène, un groupe alkyle, un groupe alcényle, un groupe alcynyle, un groupe cycloalkyle, un groupe aryle, un groupe hydroxyle, un groupe alcoxy, un atome d'halogène, un groupe C-carboxyle, un groupe O-carboxyle, un groupe carbonyle, un groupe thiocarbonyle, un groupe C-amido, un groupe guanyle, un groupe sulfonyle, un groupe trihalogénométhane-sulfonyle et une paire d'électrons ;

R_2 est choisi parmi le groupe constitué par un atome d'hydrogène, un groupe alkyle, un groupe alcényle, un groupe alcynyle, un groupe cycloalkyle, un groupe aryle, un groupe hydroxyle, un groupe alcoxy, un atome d'halogène, un groupe C-carboxyle, un groupe 0-carboxyle, un groupe carbonyle, un groupe thiocarbonyle, un groupe C-amido, un groupe guanyle, un groupe sulfonyle et un groupe trihalogénométhane-sulfonyle ; ou bien, en variante R_1 et R_2 ou R_2 et R_3 forment une structure à noyau pentagonal à heptagonal ;

R_6 est choisi parmi le groupe constitué par un groupe alkyle, un groupe trihalogénoalkyle, un groupe cycloalkyle, un groupe alcényle, un groupe alcynyle, un groupe aryle, un groupe hétéroaryle, un groupe hétéro-alicyclique, un groupe hydroxyle, un groupe alcoxy, un groupe aryloxy, un groupe thiohydroxyle, un groupe thio-alcoxy, un groupe thio-aryloxy, un groupe sulfinyle, un groupe sulfonyle, un groupe N-sulfonamido, un groupe S-sulfonamido, un groupe trihalogénométhylsulfonamido, un groupe carbonyle, un groupe thiocarbonyle, un groupe C-carboxyle, un groupe O-carboxyle, un groupe C-amido, un groupe N-amido, un groupe cyano, un groupe nitro, un groupe halogéno, un groupe O-carbamyle, un groupe N-carbamyle, un groupe O-thiocarbamyle, un groupe N-thiocarbamyle, un groupe uréido, un groupe guanyle, un groupe guanidino, un groupe amino ; et un de ses sels physiologiquement acceptables ;

R_4 et R_8 sont choisis, chacun indépendamment l'un de l'autre, parmi le groupe constitué par un atome d'hydrogène, un groupe alkyle, un groupe trihalogénoalkyle, un groupe cycloalkyle, un groupe alcényle, un groupe alcynyle, un groupe aryle, un groupe hétéroaryle, un groupe hétéro-alicyclique, un groupe hydroxyle, un groupe alcoxy, un groupe aryloxy, un groupe thiohydroxyle, un groupe thio-alcoxy, un groupe thioaryloxy, un groupe sulfinyle, un groupe sulfonyle, un groupe N-sulfonamido, un groupe S-sulfonamido, un groupe trihalogénométhylsulfonamido, un groupe carbonyle, un groupe thiocarbonyle, un groupe C-carboxyle, un groupe O-carboxyle, un groupe C-amido, un groupe N-amido, un groupe cyano, un groupe nitro, un groupe halogéno, un groupe O-carbamyle, un groupe N-carbamyle, un groupe O-thiocarbamyle, un groupe N-thiocarbamyle, un groupe uréido, un groupe guanyle, un groupe guanidino, un groupe amino, et un groupe $-NR_{10}R_{11}$; et un de ses sels physiologiquement acceptables ;

R_{10} et R_{11} sont choisis, chacun indépendamment l'un de l'autre, parmi le groupe constitué par un atome d'hydrogène, un groupe alkyle, un groupe cycloalkyle, un groupe aryle, un groupe carbonyle et un groupe sulfonyle, ou bien, en variante, R_{10} et R_{11} forment un noyau hétéro-alicyclique pentagonal ou hexagonal ; et un de ses sels physiologiquement acceptables ;

tandis que ledit radical R_6 se trouve en position 6 ;

avec cette réserve que, pour le composé I,

l'isomère de tyrphostine est capable d'inhiber l'activité de kinase du récepteur PDGF (= facteur de croissance dérivé des plaquettes).

2. Isomère purifié de tyrphostine selon la revendication 1, dans lequel

la liaison A--B est une liaison double ;

R_1 et R_2 sont choisis, chacun indépendamment l'un de l'autre, parmi le groupe constitué par un groupe alkyle, un groupe alcoxy, un atome d'halogène, un groupe aryle, un groupe hétéroaryle, un groupe nitro et un groupe amino ou bien, en variante, R_1 et R_2 forment une structure à noyau pentagonal à heptagonal ;

R_3, R_5 et R_7 représentent chacun une paire d'électrons ;

R_6 représente un groupe aryle choisi parmi le groupe constitué par un groupe phényle, un groupe ferrocène, un groupe thiophène, un groupe furanne, un groupe pyrrole, un groupe indole, un groupe thiazole, un groupe imidazole et un groupe pyridine.

3. Isomère purifié de tyrphostine selon la revendication 2, dans lequel

R_1 et R_2 représentent chacun un groupe méthyle ;

R_4 et R_8 représentent chacun un atome d'hydrogène.

4. Composition pharmaceutique comprenant, à titre d'ingrédient actif, l'isomère purifié de tyrphostine selon la revendication 1 et un support pharmaceutiquement acceptable.

5. Composition pharmaceutique selon la revendication 4, dans laquelle ledit support pharmaceutiquement acceptable est un support à libération retardée.

6. Composition pharmaceutique selon la revendication 5, dans laquelle ledit support à libération retardée est l'acide polylactique.

7. Composition pharmaceutique selon la revendication 4, à utiliser comme médicament pour traiter ou prévenir un trouble lié à la tyrosine kinase protéique dans un organisme, comprenant l'étape consistant à administrer audit organisme une quantité thérapeutiquement efficace de ladite composition pharmaceutique.

8. Utilisation d'une composition pharmaceutique selon la revendication 4, pour préparer un médicament destiné à traiter ou à prévenir un trouble lié à la tyrosine kinase protéique dans un organisme, comprenant l'étape consistant à administrer audit organisme une quantité thérapeutiquement efficace de ladite composition pharmaceutique.

9. Composition pharmaceutique selon la revendication 7 ou 8, dans laquelle ledit trouble lié à la tyrosine kinase est choisi parmi le groupe constitué par un trouble lié au EGF (= facteur de croissance de l'épiderme), un trouble lié au PDGF, un trouble lié au EGF, un trouble lié au PDGF, un trouble lié au IGF (= facteur de croissance insulino-mimétique) et un trouble lié à MET (= la méthionine).

10. Composition pharmaceutique selon la revendication 7 ou 8, dans laquelle ledit trouble lié à la tyrosine kinase est choisi parmi le groupe constitué par un trouble lié à la prolifération cellulaire, un trouble fibrogène et un trouble métabolique.

11. Composition pharmaceutique selon la revendication 10, dans laquelle ledit trouble lié à la prolifération cellulaire est choisi parmi le groupe comprenant le papillome, le glioblastome, le sarcome de Kaposi, le mélanome, le cancer des poumons, le cancer des ovaires, le cancer de la prostate, le carcinome malphigien, l'astrocytome, le cancer de la tête, le cancer du cou, le cancer de la vessie, le cancer du sein, le cancer du poumon, le cancer colorectal, le cancer de la thyroïde, le cancer pancréatique, le cancer gastrique, le cancer primitif du foie, la leucémie, le lymphome, la maladie de Hodgkin, le lymphome de Burkitt, l'arthrite, l'arthrite rhumatoïde, la rétinopathie diabétique, l'angiogenèse, la resténose, la resténose in-stent, la resténose du greffon vasculaire.

12. Composition pharmaceutique selon la revendication 10, dans laquelle ledit trouble fibrogène est choisi parmi le groupe constitué par la fibrose pulmonaire, la cirrhose du foie, l'athérosclérose, la glomérulonéphrite, la néphropathie diabétique, des syndromes de microangiopathie thrombolytique, le rejet du greffon.

13. Composition pharmaceutique selon la revendication 10, dans laquelle ledit trouble métabolique est choisi parmi le groupe constitué par le psoriasis, le diabète, la cicatrisation, l'inflammation et des maladies neurodégénératives.

14. Composition pharmaceutique selon la revendication 7 ou 8, dans laquelle ledit trouble lié à la protéine kinase est choisi parmi le groupe constitué par le papillome, le glioblastome, le sarcome de Kaposi, le mélanome, le cancer des poumons, le cancer des ovaires, le cancer de la prostate, le carcinome malphigien, l'astrocytome, le cancer de la tête, le cancer du cou, le cancer de la vessie, le cancer du sein, le cancer bronchique à petites cellules, le cancer colorectal, le cancer de la thyroïde, le cancer pancréatique, le cancer gastrique, le cancer primitif du foie, la leucémie, le lymphome, la maladie de Hodgkin, le lymphome de Burkitt, le psoriasis, la fibrose pulmonaire, l'arthrite, l'arthrite rhumatoïde, la rétinopathie diabétique, la resténose, la resténose in-stent, la resténose du greffon vasculaire, la cirrhose du foie, l'athérosclérose, l'angiogenèse, la glomérulonéphrite, la néphropathie diabétique, des syndromes de microangiopathie thrombotique, le rejet du greffon, les maladies auto-immunes, la cicatrisation, l'inflammation, les maladies neurodégénératives, le diabète et les troubles hyperimmuns.

15. Composition pharmaceutique selon la revendication 7 ou 8, dans laquelle ledit organisme est un mammifère.

16. Composition pharmaceutique selon la revendication 15, dans laquelle ledit mammifère est un être humain.

17. Composition pharmaceutique selon la revendication 4, à utiliser comme médicament pour le traitement local ou la prévention locale d'un trouble tissulaire d'un organisme comprenant l'étape consistant à appliquer localement ladite composition pharmaceutique sur ledit tissu.

18. Utilisation d'une composition pharmaceutique selon la revendication 4, pour la préparation d'un médicament destiné au traitement local ou à la prévention locale d'un trouble tissulaire d'un organisme comprenant l'étape consistant à appliquer localement ladite composition pharmaceutique sur ledit tissu.

19. Composition pharmaceutique selon la revendication 17 ou 18, dans laquelle ledit organisme est un être humain.

20. Composition pharmaceutique selon la revendication 17 ou 18, dans laquelle ledit tissu est choisi parmi le groupe constitué par un vaisseau sanguin, le poumon et la peau.

21. Isomère purifié de tyrphostine selon la revendication 1, à utiliser comme médicament pour inhiber la prolifération cellulaire, comprenant l'étape consistant à exposer des cellules audit isomère purifié de tyrphostine.

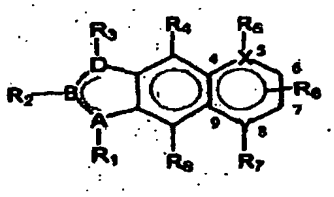
22. Utilisation d'une composition pharmaceutique selon la revendication 4, pour la préparation d'un médicament destiné à inhiber la prolifération cellulaire, comprenant l'étape consistant à exposer des cellules audit isomère purifié de tyrphostine.

23. Isomère purifié de tyrphostine selon la revendication 21 ou 22, dans lequel lesdites cellules sont des cellules d'un organisme, tandis que l'exposition des cellules audit isomère purifié de tyrphostine est mise en oeuvre in vivo.

24. Isomère purifié de tyrphostine selon la revendication 23, dans lequel ledit organisme est un être humain.

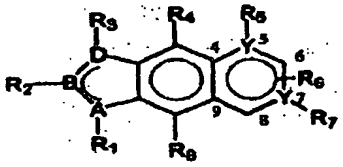
25. Isomère purifié de tyrphostine selon la revendication 21 ou 22, dans lequel l'exposition des cellules audit isomère purifié de tyrphostine est mise en oeuvre in vivo.

26. Procédé d'enregistrement, pour un isomère spécifique, d'une tyrphostine répondant à la formule générale



(composé I)

ou



(composé II)

dans laquelle

les chiffres 4, 5, 6, 7, 8 et 9 indiquent des positions sur un noyau aromatique hexagonal terminal ;

A, D, X et Y représentent chacun un atome d'azote ;

B représente un atome de carbone ;

la ligne en pointillé dans le noyau pentagonal signifie que, soit la liaison A--B, soit la liaison B--D est une liaison double ;

lorsque A ou D représente un atome d'azote et lorsque ledit atome d'azote fait partie d'une liaison double, R_1 ou R_3, respectivement représente une paire d'électrons ;

lorsque A ou D représente un atome d'azote et lorsque ledit atome d'azote ne fait pas partie d'une liaison double, R_1 ou R_3 est choisi parmi le groupe constitué par un groupe alkyle, un groupe alcényle, un groupe alcynyle, un groupe cycloalkyle, un groupe aryle, un groupe hydroxyle, un groupe alcoxy, un atome d'halogène, un groupe C-carboxyle, un groupe O-carboxyle, un groupe carbonyle, un groupe thiocarbonyle, un groupe C-amido, un groupe guanyle, un groupe sulfonyle et un groupe trihalogénométhane-sulfonyle ;

R_5 et R_7 sont choisis, chacun indépendamment l'un de l'autre, parmi le groupe constitué par un atome d'hydrogène, un groupe alkyle, un groupe alcényle, un groupe alcynyle, un groupe cycloalkyle, un groupe aryle, un

groupe hydroxyle, un groupe alcoxy, un atome d'halogène, un groupe C-carboxyle, un groupe O-carboxyle, un groupe carbonyle, un groupe thiocarbonyle, un groupe C-amido, un groupe guanyle, un groupe sulfonyle, un groupe trihalogénométhane-sulfonyle et une paire d'électrons ;

R_2 est choisi parmi le groupe constitué par un atome d'hydrogène, un groupe alkyle, un groupe alcényle, un groupe alcynyle, un groupe cycloalkyle, un groupe aryle, un groupe hydroxyle, un groupe alcoxy, un atome d'halogène, un groupe C-carboxyle, un groupe O-carboxyle, un groupe carbonyle, un groupe thiocarbonyle, un groupe C-amido, un groupe guanyle, un groupe sulfonyle et un groupe trihalogénométhane-sulfonyle ; ou bien, en variante R_1 et R_2 ou R_2 et R_3 forment une structure à noyau pentagonal à heptagonal ;

R_6 est choisi parmi le groupe constitué par un groupe alkyle, un groupe trihalogénoalkyle, un groupe cycloalkyle, un groupe alcényle, un groupe alcynyle, un groupe aryle, un groupe hétéroaryle, un groupe hétéro-alicyclique, un groupe hydroxyle, un groupe alcoxy, un groupe aryloxy, un groupe thiohydroxyle, un groupe thio-alcoxy, un groupe thio-aryloxy, un groupe sulfinyle, un groupe sulfonyle, un groupe N-sulfonamido, un groupe S-sulfonamido, un groupe trihalogénométhylsulfonamido, un groupe carbonyle, un groupe thiocarbonyle, un groupe C-carboxyle, un groupe O-carboxyle, un groupe C-amido, un groupe N-amido, un groupe cyano, un groupe nitro, un groupe halogéno, un groupe O-carbamyle, un groupe N-carbamyle, un groupe O-thiocarbamyle, un groupe N-thiocarbamyle, un groupe uréido, un groupe guanyle, un groupe guanidino, un groupe amino ; et un de ses sels physiologiquement acceptables ou un de ses promédicaments ;

R_4 et R_8 sont choisis, chacun indépendamment l'un de l'autre, parmi le groupe constitué par un atome d'hydrogène, un groupe alkyle, un groupe trihalogénoalkyle, un groupe cycloalkyle, un groupe alcényle, un groupe alcynyle, un groupe aryle, un groupe hétéroaryle, un groupe hétéro-alicyclique, un groupe hydroxyle, un groupe alcoxy, un groupe aryloxy, un groupe thiohydroxyle, un groupe thio-alcoxy, un groupe thioaryloxy, un groupe sulfinyle, un groupe sulfonyle, un groupe N-sulfonamido, un groupe S-sulfonamido, un groupe trihalogénométhylsulfonamido, un groupe carbonyle, un groupe thiocarbonyle, un groupe C-carboxyle, un groupe O-carboxyle, un groupe C-amido, un groupe N-amido, un groupe cyano, un groupe nitro, un groupe halogéno, un groupe O-carbamyle, un groupe N-carbamyle, un groupe O-thiocarbamyle, un groupe N-thiocarbamyle, un groupe uréido, un groupe guanyle, un groupe guanidino, un groupe amino, et un groupe $-NR_{10}R_{11}$; et un de ses sels physiologiquement acceptables ou un de ses promédicaments ;

R_{10} et R_{11} sont choisis, chacun indépendamment l'un de l'autre, parmi le groupe constitué par un atome d'hydrogène, un groupe alkyle, un groupe cycloalkyle, un groupe aryle, un groupe carbonyle et un groupe sulfonyle, ou bien, en variante, R_{10} et R_{11} forment un noyau hétéro-alicyclique pentagonal ou hexagonal ; et un de ses sels physiologiquement acceptables ou un de ses promédicaments ;

tandis que, pour chaque molécule du composé I, le radical R_6 se trouve en position 6 ou 7, ou bien, pour chaque molécule du composé II, le radical R_6 se trouve en position 6 ou 8 ;

l'isomère de tyrphostine étant à même d'inhiber l'activité de kinase du récepteur de PDGF ;

le procédé comprenant les étapes consistant à :

(i) chromatographier ladite tyrphostine à travers une matrice pour ainsi séparer les isomères de position de ladite tyrphostine ;

(ii) récolter au moins un isomère de position spécifique de ladite tyrphostine.

27. Procédé selon la revendication 26, comprenant en outre l'étape consistant à :

(iii) cristalliser ledit au moins un isomère de position spécifique.

28. Procédé selon la revendication 26, dans lequel la liaison A--B est une liaison double ;

R_1 et R_2 sont choisis, chacun indépendamment l'un de l'autre, parmi le groupe constitué par un groupe alkyle, un groupe alcoxy, un atome d'halogène, un groupe aryle, un groupe hétéroaryle, un groupe nitro et un groupe amino ou bien, en variante, R_1 et R_2 forment une structure de noyau pentagonal à heptagonal ; R_3, R_5 et R_7 représentent chacun une paire d'électrons ;

R_6 représente un groupe aryle choisi parmi le groupe constitué par un groupe phényle, un groupe ferrocène, un groupe thiophène, un groupe furanne, un groupe pyrrole, un groupe indole, un groupe thiazole, un groupe imidazole et un groupe pyridine.

29. Procédé selon la revendication 28, dans lequel

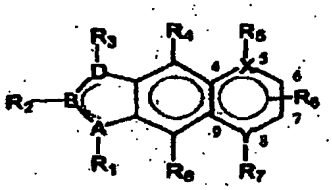
R_1 et R_2 représentent chacun un groupe méthyle ;

R_4 et R_8 représentent chacun un atome d'hydrogène.

30. Procédé pour préparer une composition pharmaceutique pour une libération retardée d'une tyrphostine, compre-

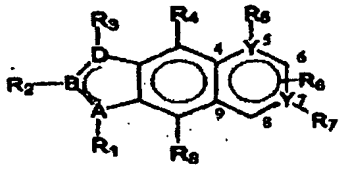
nant les étapes consistant à :

(i) procurer un isomère purifié de tyrphostine répondant à la formule générale :



(composé I)

ou



(composé II)

dans laquelle

les chiffres 4, 5, 6, 7, 8 et 9 indiquent des positions sur un noyau aromatique hexagonal terminal ;

A, D, X et Y représentent chacun un atome d'azote ;

la ligne en pointillé dans le noyau pentagonal signifie que, soit la liaison A--B, soit la liaison B--D est une liaison double ;

lorsque A ou D représente un atome d'azote et lorsque ledit atome d'azote fait partie d'une liaison double, R_1 ou R_3, respectivement représente une paire d'électrons ;

lorsque A ou D représente un atome d'azote et lorsque ledit atome d'azote ne fait pas partie d'une liaison double, R_1 ou R_3 est choisi parmi le groupe constitué par un groupe alkyle, un groupe alcényle, un groupe alcynyle, un groupe cycloalkyle, un groupe aryle, un groupe hydroxyle, un groupe alcoxy, un atome d'halogène, un groupe C-carboxyle, un groupe O-carboxyle, un groupe carbonyle, un groupe thiocarbonyle, un groupe C-amido, un groupe guanyle, un groupe sulfonyle et un groupe trihalogénométhane-sulfonyle ;

R_5 et R_7 sont choisis, chacun indépendamment l'un de l'autre, parmi le groupe constitué par un atome d'hydrogène, un groupe alkyle, un groupe alcényle, un groupe alcynyle, un groupe cycloalkyle, un groupe aryle, un groupe hydroxyle, un groupe alcoxy, un atome d'halogène, un groupe C-carboxyle, un groupe O-carboxyle, un groupe carbonyle, un groupe thiocarbonyle, un groupe C-amido, un groupe guanyle, un groupe sulfonyle, un groupe trihalogénométhane-sulfonyle et une paire d'électrons ;

R_2 est choisi parmi le groupe constitué par un atome d'hydrogène, un groupe alkyle, un groupe alcényle, un groupe alcynyle, un groupe cycloalkyle, un groupe aryle, un groupe hydroxyle, un groupe alcoxy, un atome d'halogène, un groupe C-carboxyle, un groupe O-carboxyle, un groupe carbonyle, un groupe thiocarbonyle, un groupe C-amido, un groupe guanyle, un groupe sulfonyle et un groupe trihalogénométhane-sulfonyle ; ou bien,

en variante R_1 et R_2 ou R_2 et R_3 forment une structure à noyau pentagonal à heptagonal ;

R_6 est choisi parmi le groupe constitué par un groupe alkyle, un groupe trihalogénoalkyle, un groupe cycloalkyle, un groupe alcényle, un groupe alcynyle, un groupe aryle, un groupe hétéroaryle, un groupe hétéroalicyclique, un groupe hydroxyle, un groupe alcoxy, un groupe aryloxy, un groupe thiohydroxyle, un groupe thio-alcoxy, un groupe thio-aryloxy, un groupe sulfinyle, un groupe sulfonyle, un groupe N-sulfonamido, un groupe S-sulfonamido, un groupe trihalogénométhylsulfonamido, un groupe carbonyle, un groupe thiocarbonyle, un groupe C-carboxyle, un groupe O-carboxyle, un groupe C-amido, un groupe N-amido, un groupe cyano, un groupe nitro, un groupe halogéno, un groupe O-carbamyle, un groupe N-carbamyle, un groupe O-thiocarbamyle, un groupe N-thiocarbamyle, un groupe uréido, un groupe guanyle, un groupe guanidino, un groupe amino ; et un de ses sels physiologiquement acceptables ou un de ses promédicaments ;

R_4 et R_8 sont choisis, chacun indépendamment l'un de l'autre, parmi le groupe constitué par un atome d'hydrogène, un groupe alkyle, un groupe trihalogénoalkyle, un groupe cycloalkyle, un groupe alcényle, un

groupe alcynyle, un groupe aryle, un groupe hétéroaryle, un groupe hétéro-alicyclique, un groupe hydroxyle, un groupe alcoxy, un groupe aryloxy, un groupe thiohydroxyle, un groupe thio-alcoxy, un groupe thioaryloxy, un groupe sulfinyle, un groupe sulfonyle, un groupe N-sulfonamido, un groupe S-sulfonamido, un groupe trihalogénométhylsulfonamido, un groupe carbonyle, un groupe thiocarbonyle, un groupe C-carboxyle, un groupe O-carboxyle, un groupe C-amido, un groupe N-amido, un groupe cyano, un groupe nitro, un groupe halogéno, un groupe O-carbamyle, un groupe N-carbamyle, un groupe O-thiocarbamyle, un groupe N-thiocarbamyle, un groupe uréido, un groupe guanyle, un groupe guanidino, un groupe amino, et un groupe $-NR_{10}R_{11}$; et un de ses sels physiologiquement acceptables ou un de ses promédicaments ;

R_{10} et R_{11} sont choisis, chacun indépendamment l'un de l'autre, parmi le groupe constitué par un atome d'hydrogène, un groupe alkyle, un groupe cycloalkyle, un groupe aryle, un groupe carbonyle et un groupe sulfonyle, ou bien, en variante, R_{10} et R_{11} forment un noyau hétéro-alicyclique pentagonal ou hexagonal ; et un de ses sels physiologiquement acceptables ou un de ses promédicaments ;

tandis que, ledit radical R_6 se trouve en position 6 ;

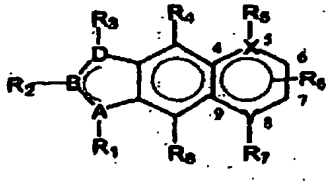
(ii) dissoudre ou disperser un support à libération retardée et ledit isomère purifié de tyrphostine dans un solvant organique pour obtenir une solution organique contenant ledit support et ledit isomère purifié de tyrphostine ;

(iii) ajouter ladite solution organique à une solution aqueuse pour obtenir une émulsion de type huile-dans-l'eau ;

(iv) évaporer ledit solvant organique de ladite émulsion de type huile-dans-l'eau pour obtenir une suspension colloïdale de particules contenant ledit support à libération retardée et ledit isomère purifié de tyrphostine.

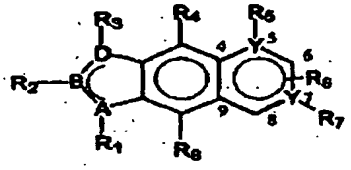
31. Procédé selon la revendication 30, dans lequel ledit support à libération retardée est l'acide polylactique.

32. Endoprothèse vasculaire comprenant un corps essentiellement tubulaire, le corps étant constitué d'une matière conçue pour la libération retardée d'un isomère purifié de tyrphostine répondant à la formule générale :



(composé I)

ou



(composé II)

dans laquelle

les chiffres 4, 5, 6, 7, 8 et 9 indiquent des positions sur un noyau aromatique hexagonal terminal ;

A, D, X et Y représentent chacun un atome d'azote ;

la ligne en pointillé dans le noyau pentagonal signifie que, soit la liaison A--B, soit la liaison B--D est une liaison double ;

lorsque A ou D représente un atome d'azote et lorsque ledit atome d'azote fait partie d'une liaison double, R_1 ou R_3, respectivement représente une paire d'électrons ;

lorsque A ou D représente un atome d'azote et lorsque ledit atome d'azote ne fait pas partie d'une liaison double, R_1 ou R_3 est choisi parmi le groupe constitué par un groupe alkyle, un groupe alcényle, un groupe alcynyle, un groupe cycloalkyle, un groupe aryle, un groupe hydroxyle, un groupe alcoxy, un atome d'halogène, un groupe C-carboxyle, un groupe O-carboxyle, un groupe carbonyle, un groupe thiocarbonyle, un groupe C-amido, un groupe guanyle, un groupe sulfonyle et un groupe trihalogénométhane-sulfonyle ;

R_5 et R_7 sont choisis, chacun indépendamment l'un de l'autre, parmi le groupe constitué par un atome d'hydrogène, un groupe alkyle, un groupe alcényle, un groupe alcynyle, un groupe cycloalkyle, un groupe aryle, un groupe hydroxyle, un groupe alcoxy, un atome d'halogène, un groupe C-carboxyle, un groupe O-carboxyle, un groupe carbonyle, un groupe thiocarbonyle, un groupe C-amido, un groupe guanyle, un groupe sulfonyle, un groupe trihalogénométhane-sulfonyle et une paire d'électrons ;

R_2 est choisi parmi le groupe constitué par un atome d'hydrogène, un groupe alkyle, un groupe alcényle, un groupe alcynyle, un groupe cycloalkyle, un groupe aryle, un groupe hydroxyle, un groupe alcoxy, un atome d'halogène, un groupe C-carboxyle, un groupe O-carboxyle, un groupe carbonyle, un groupe thiocarbonyle, un groupe C-amido, un groupe guanyle, un groupe sulfonyle et un groupe trihalogénométhane-sulfonyle ; ou bien, en variante R_1 et R_2 ou R_2 et R_3 forment une structure à noyau pentagonal à heptagonal ;

R_6 est choisi parmi le groupe constitué par un groupe alkyle, un groupe trihalogénoalkyle, un groupe cycloalkyle, un groupe alcényle, un groupe alcynyle, un groupe aryle, un groupe hétéroaryle, un groupe hétéro-alicyclique, un groupe hydroxyle, un groupe alcoxy, un groupe aryloxy, un groupe thiohydroxyle, un groupe thio-alcoxy, un groupe thio-aryloxy, un groupe sulfinyle, un groupe sulfonyle, un groupe N-sulfonamido, un groupe S-sulfonamido, un groupe trihalogénométhylsulfonamido, un groupe carbonyle, un groupe thiocarbonyle, un groupe C-carboxyle, un groupe O-carboxyle, un groupe C-amido, un groupe N-amido, un groupe cyano, un groupe nitro, un groupe halogéno, un groupe O-carbamyle, un groupe N-carbamyle, un groupe O-thiocarbamyle, un groupe N-thiocarbamyle, un groupe uréido, un groupe guanyle, un groupe guanidino, un groupe amino ; et un de ses sels physiologiquement acceptables ou un de ses promédicaments ;

R_4 et R_8 sont choisis, chacun indépendamment l'un de l'autre, parmi le groupe constitué par un atome d'hydrogène, un groupe alkyle, un groupe trihalogénoalkyle, un groupe cycloalkyle, un groupe alcényle, un groupe alcynyle, un groupe aryle, un groupe hétéroaryle, un groupe hétéro-alicyclique, un groupe hydroxyle, un groupe alcoxy, un groupe aryloxy, un groupe thiohydroxyle, un groupe thio-alcoxy, un groupe thioaryloxy, un groupe sulfinyle, un groupe sulfonyle, un groupe N-sulfonamido, un groupe S-sulfonamido, un groupe trihalogénométhylsulfonamido, un groupe carbonyle, un groupe thiocarbonyle, un groupe C-carboxyle, un groupe O-carboxyle, un groupe C-amido, un groupe N-amido, un groupe cyano, un groupe nitro, un groupe halogéno, un groupe O-carbamyle, un groupe N-carbamyle, un groupe O-thiocarbamyle, un groupe N-thiocarbamyle, un groupe uréido, un groupe guanyle, un groupe guanidino, un groupe amino, et un groupe $-NR_{10}R_{11}$; et un de ses sels physiologiquement acceptables ou un de ses promédicaments ;

R_{10} et R_{11} sont choisis, chacun indépendamment l'un de l'autre, parmi le groupe constitué par un atome d'hydrogène, un groupe alkyle, un groupe cycloalkyle, un groupe aryle, un groupe carbonyle et un groupe sulfonyle, ou bien, en variante, R_{10} et R_{11} forment un noyau hétéro-alicyclique pentagonal ou hexagonal ; et un de ses sels physiologiquement acceptables ou un de ses promédicaments ;

tandis que, ledit radical R_6 se trouve en position 6 ;

l'isomère de tyrphostine étant à même d'inhiber l'activité de kinase du récepteur du PDGF.

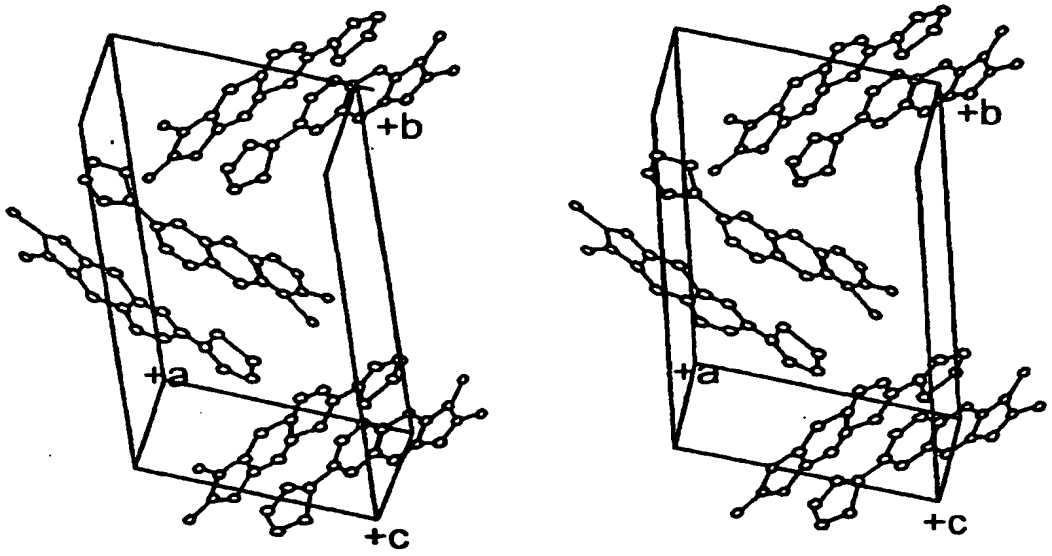


Fig. 1

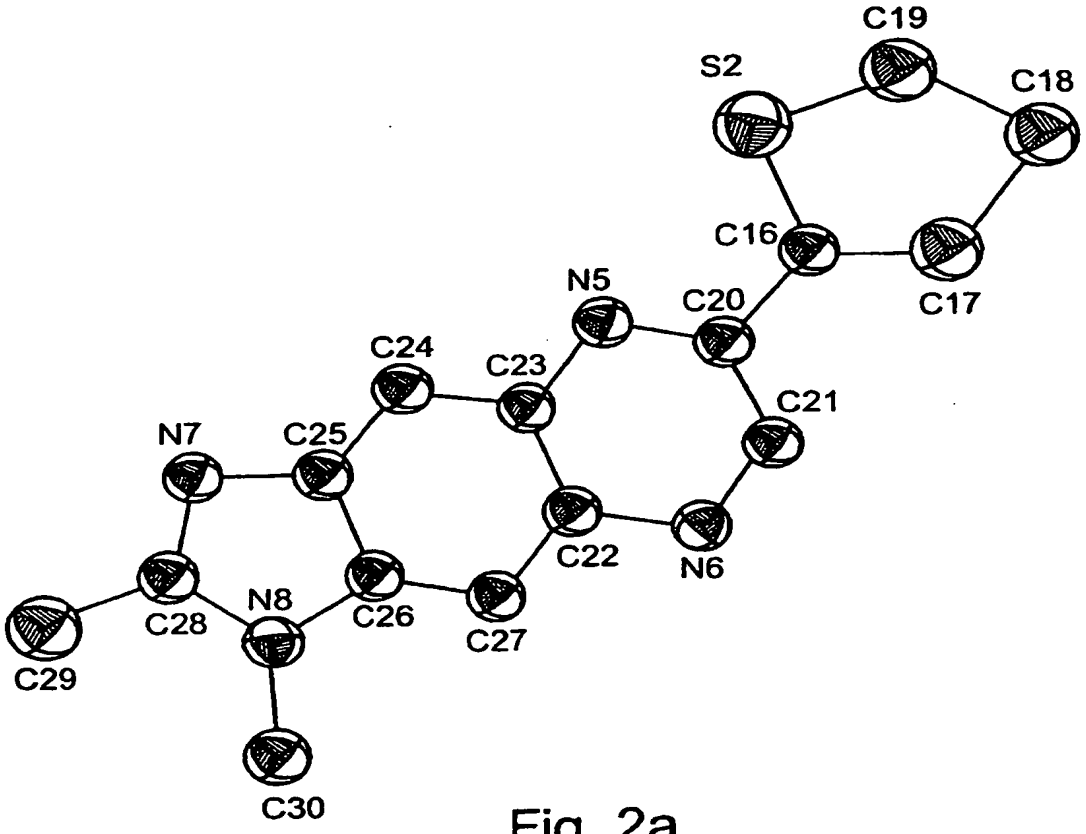

C19
S2
C18
C16
C17
N5
C20
C24
C23
C21
N7
C25
C22
N6
N8
C28
C26
C27
C29
C30

Fig. 2a

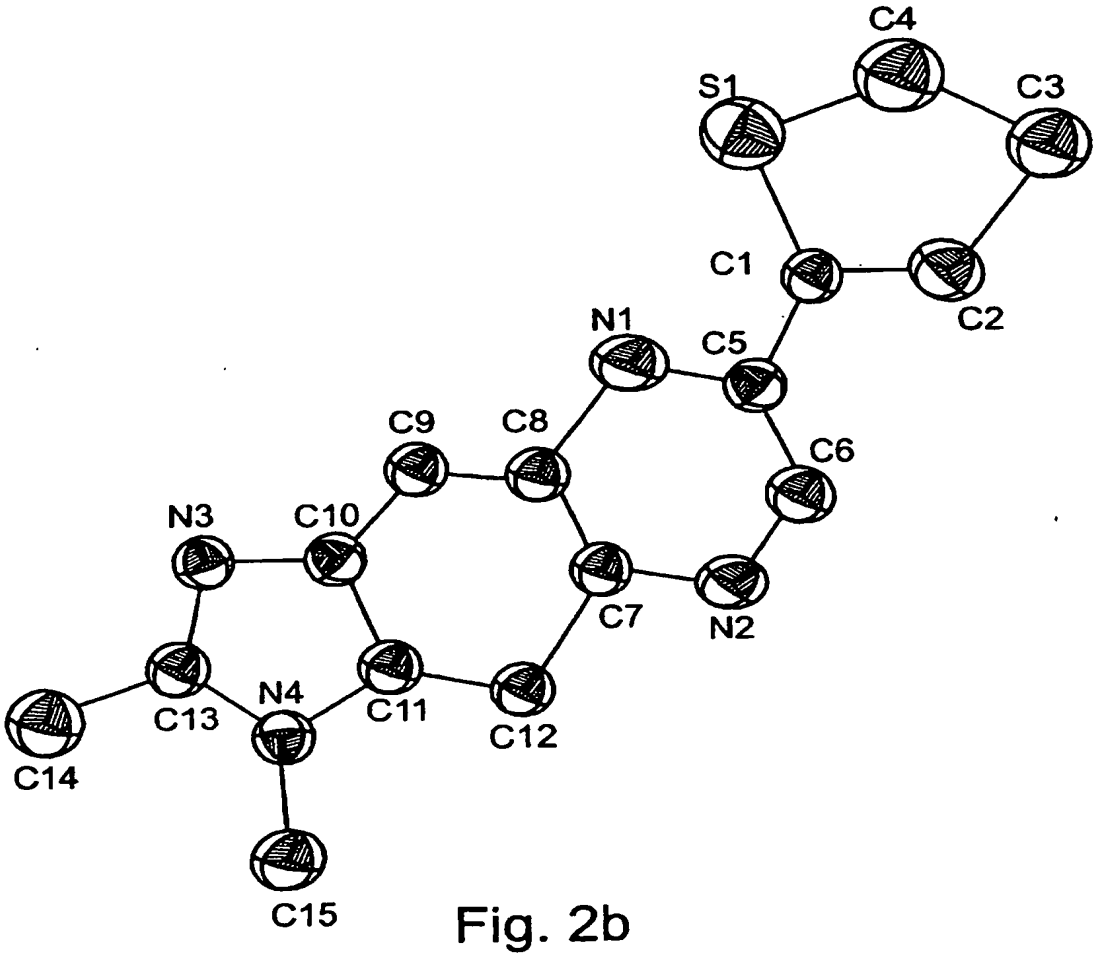


Fig. 2b

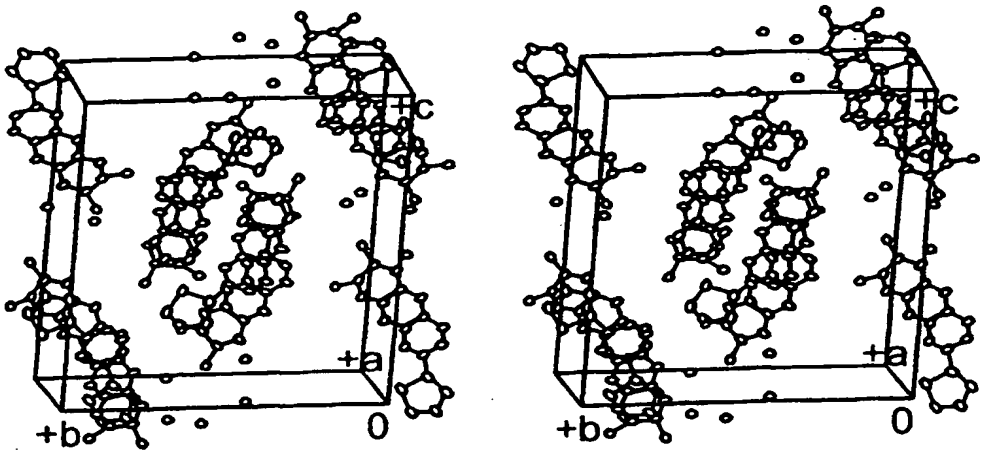


Fig. 3

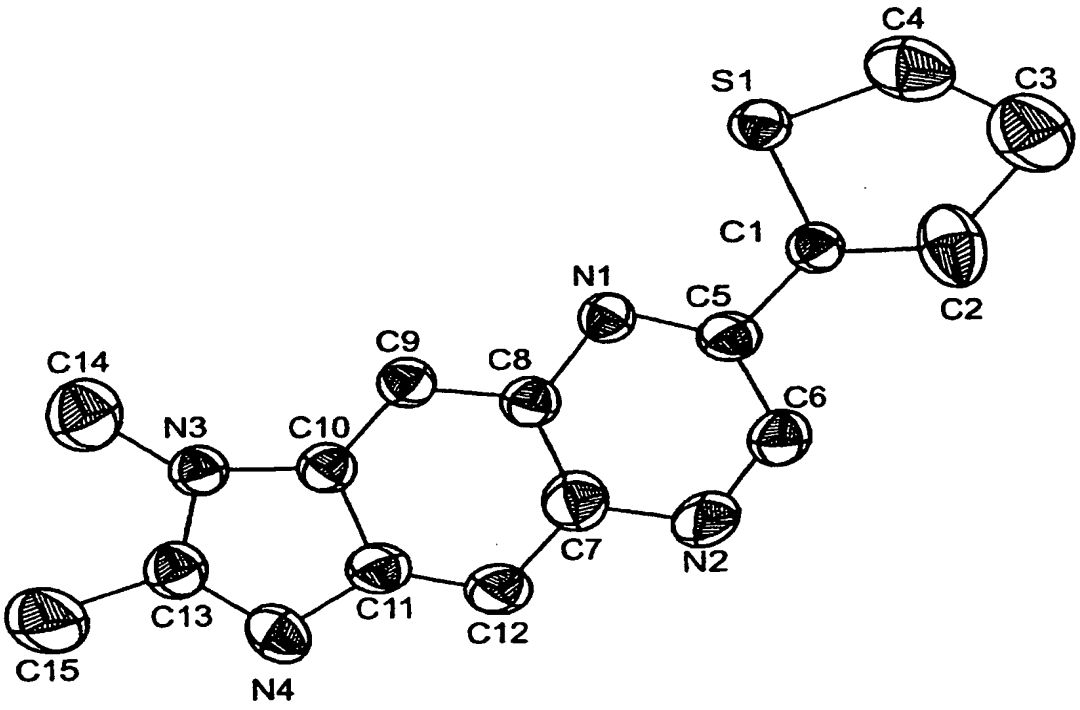


Fig. 4a

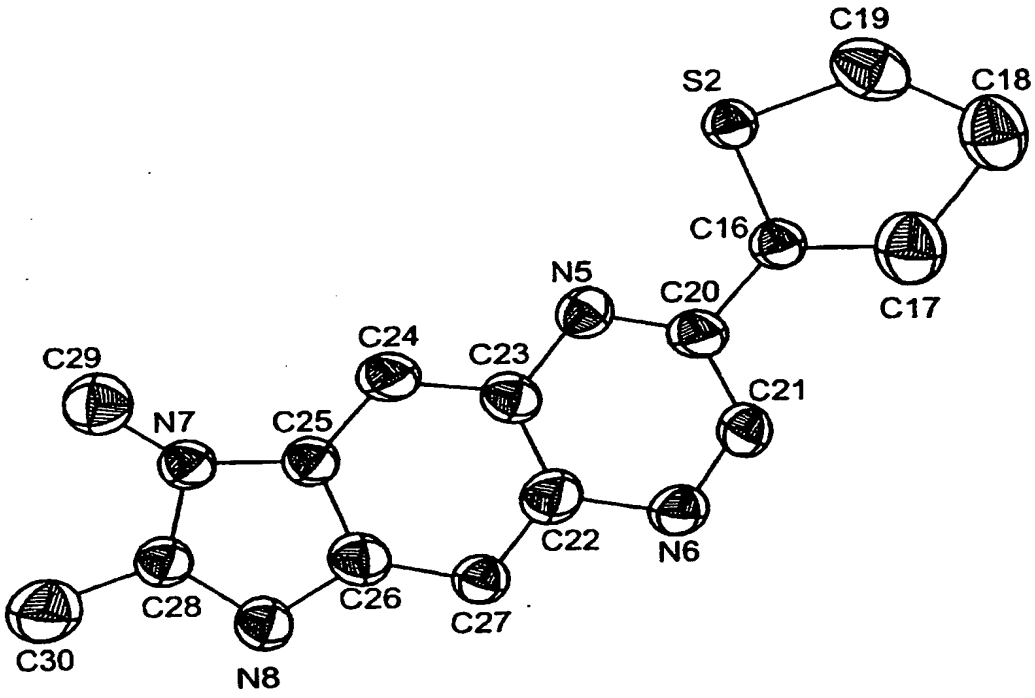

C19
S2
C18
C16
C17
N5
C20
C24
C23
C29
C21
N7
C25
C22
N6
C28
C26
C27
C30
N8

Fig. 4b

In vitro kinase assay

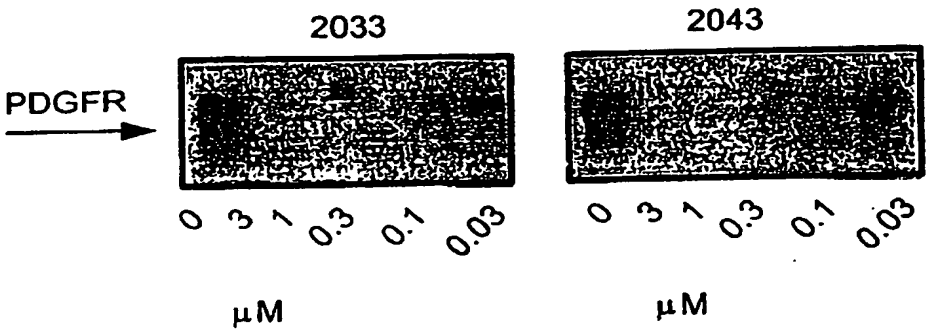


Fig. 5

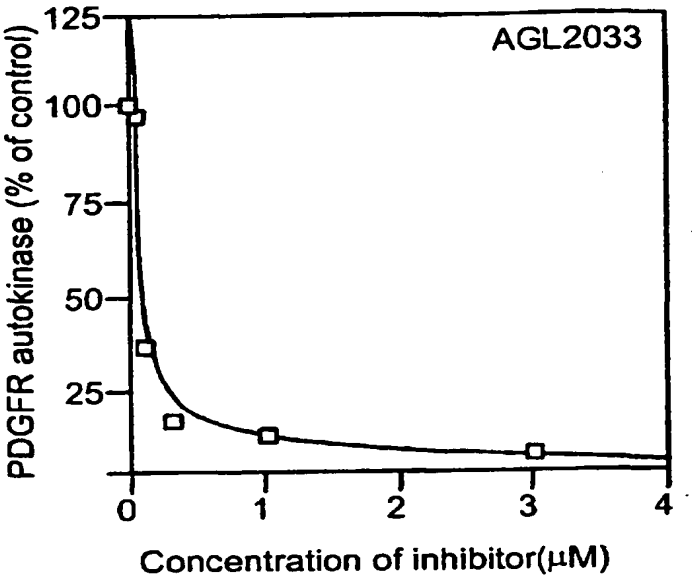

AGL2033
PDGFR autokinase (% of control)
125
100
75
50
25
0
1
2
3
4
Concentration of inhibitor(μM)

Fig. 6

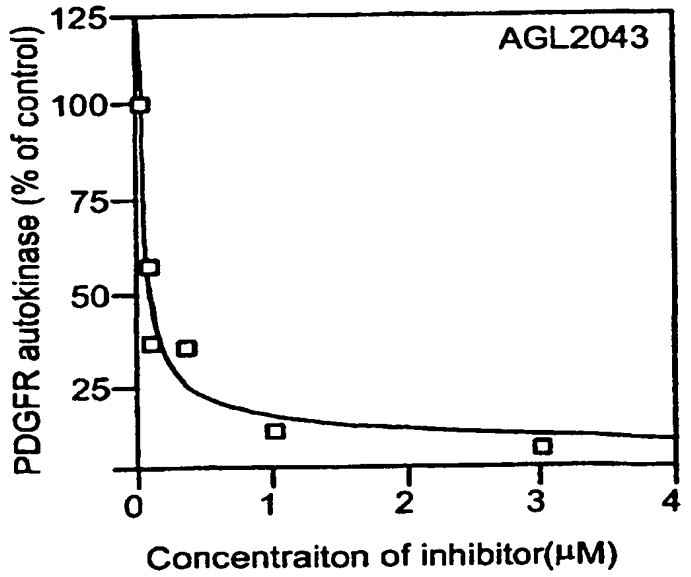

AGL2043
PDGFR autokinase (% of control)
125
100
75
50
25
0
1
2
3
4
Concentraiton of inhibitor(μM)

Fig. 7

Swiss 3T3 cells

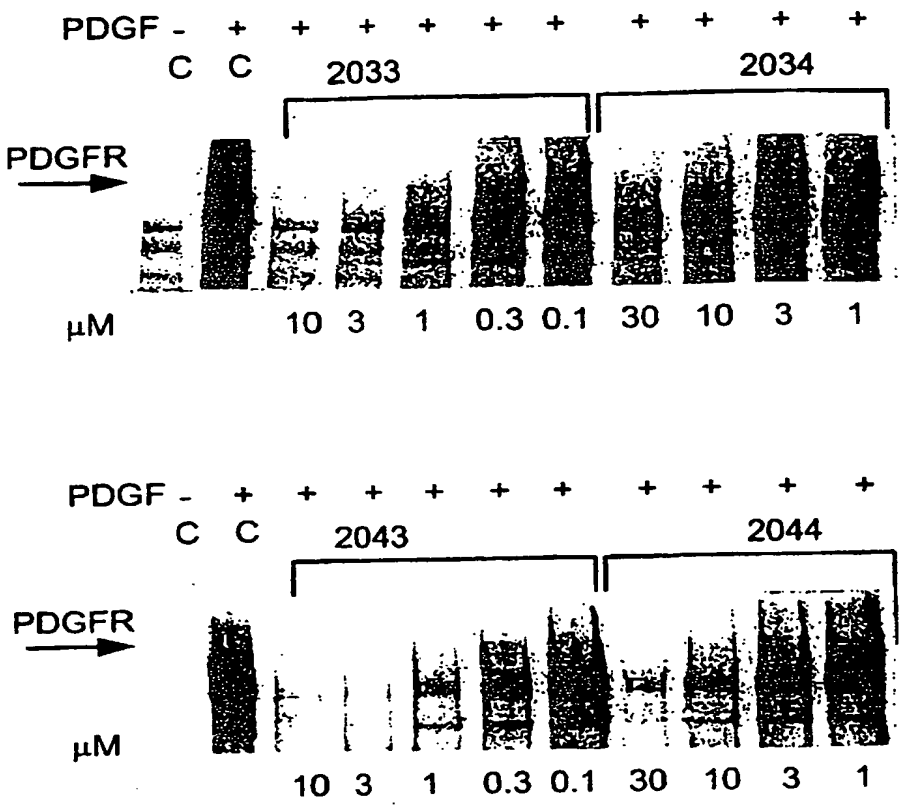


Fig. 8

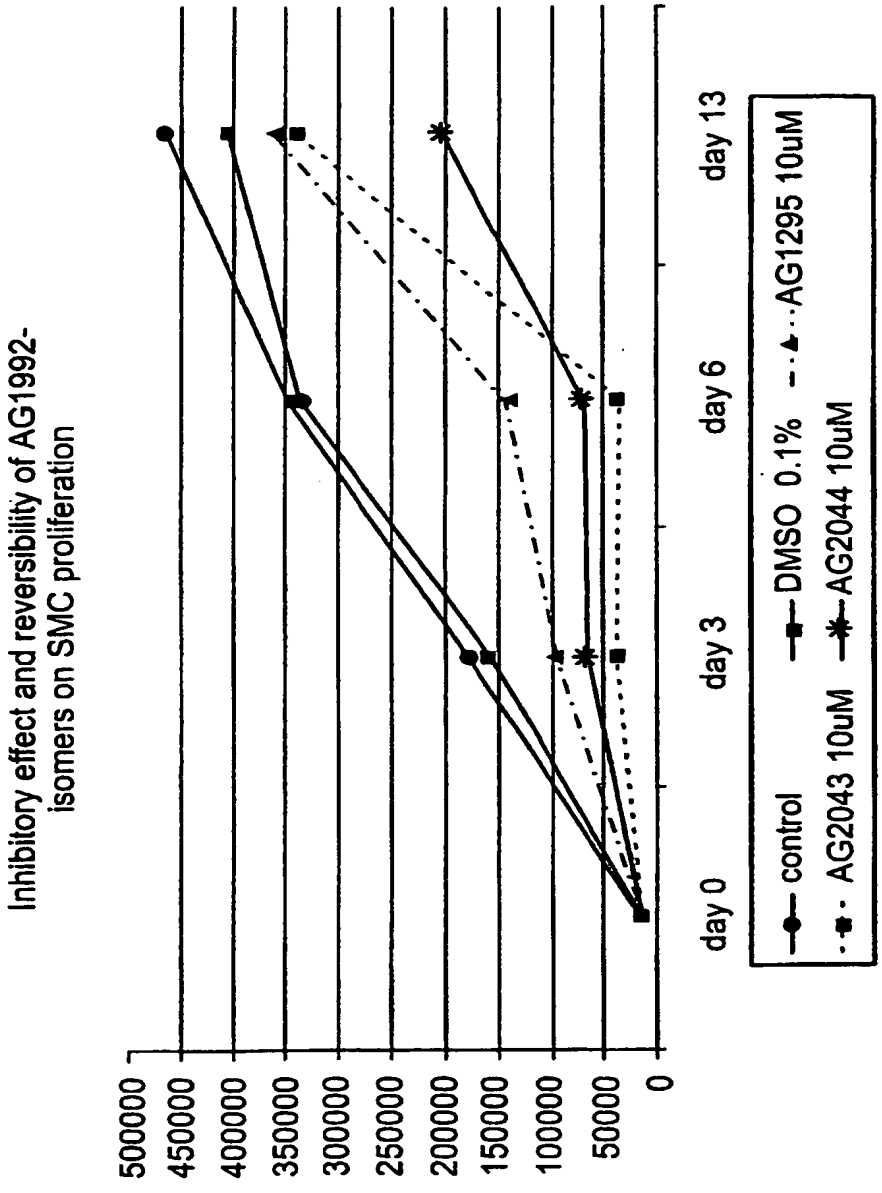

Inhibitory effect and reversibility of AG1992-isomers on SMC proliferation
500000
450000
400000
350000
300000
250000
200000
150000
100000
50000
0
day 0
day 3
day 6
day 13
control
DMSO 0.1%
AG1295 10uM
AG2043 10uM
AG2044 10uM

Fig. 9

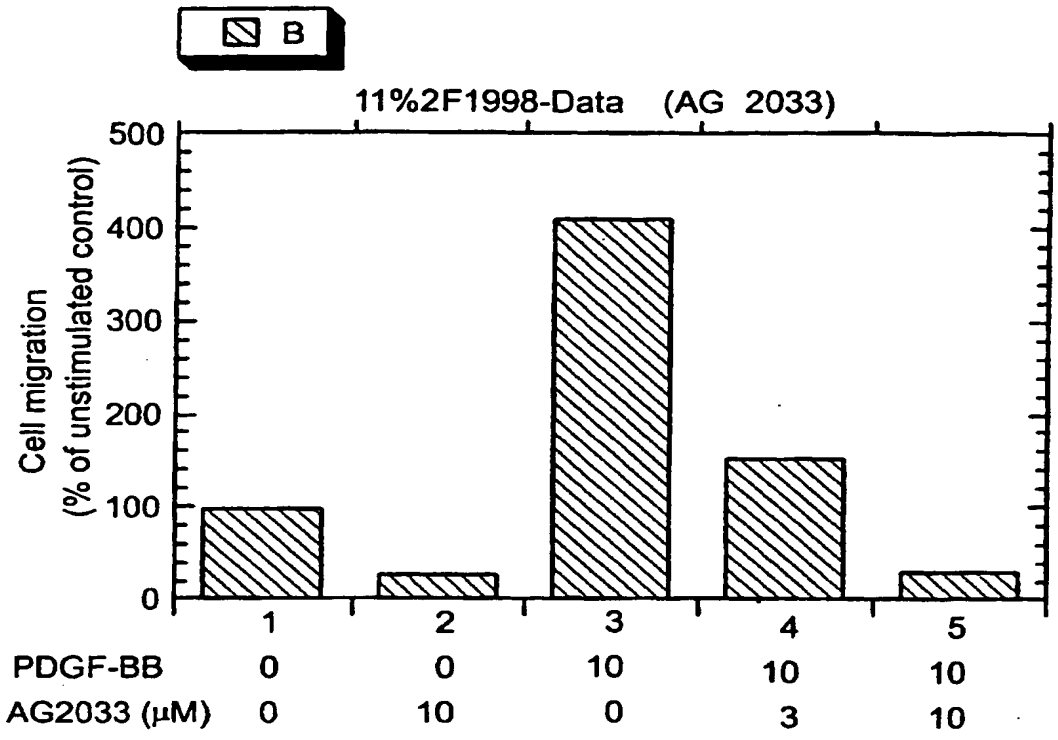

B
11%2F1998-Data (AG 2033)
Cell migration
(% of unstimulated control)
500
400
300
200
100
0
1
2
3
4
5
PDGF-BB
0
0
10
10
10
AG2033 (µM)
0
10
0
3
10

Fig. 10

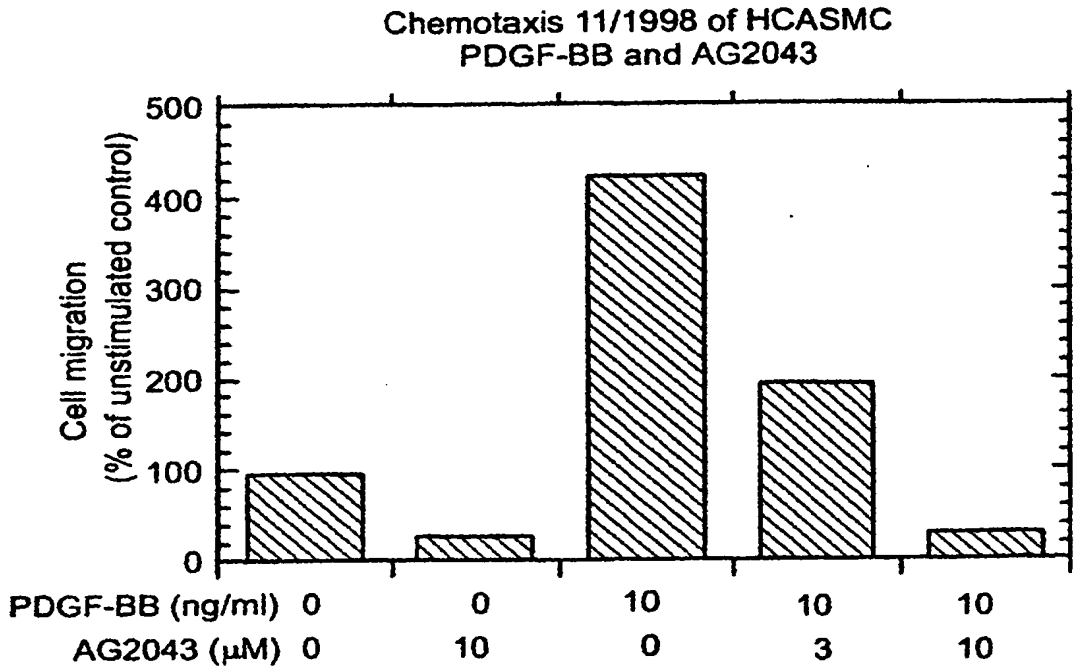

Chemotaxis 11/1998 of HCASMC
PDGF-BB and AG2043
Cell migration
(% of unstimulated control)
500
400
300
200
100
0
PDGF-BB (ng/ml) 0 0 10 10 10
AG2043 (µM) 0 10 0 3 10

Fig. 11